HINDUSTAN ANTIBIOTICS

Bulletin

NOVEMBER 1961

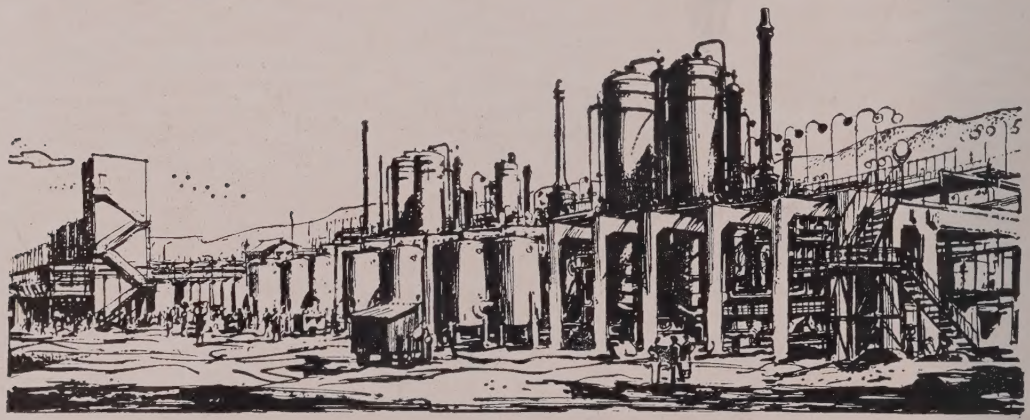
VOL. 4

2

NOW made at Trombay by UNION CARBIDE INDIA

UNION CARBIDE TRADE MARK

Butyl Acetate



Check these qualities of "Union Carbide" Butyl Acetate and you will agree you cannot buy a more versatile medium-boiling solvent for nitrocellulose.

PERMITS FORMULATION OF HIGH QUALITY LACQUERS WITH OUTSTANDING VISCOSITY CHARACTERISTICS

ENSURES FREEDOM FROM MOISTURE BLUSH

NO ORANGE PEEL, SAG OR TACKINESS

Often considered a standard for solvent comparisons by the lacquer industry, "Union Carbide" Butyl Acetate is also widely used in the manufacture of dopes, artificial leather, photographic films, printing inks, adhesives and paper coatings, and as an extractant in antibiotic manufacture.

For your requirements of Butyl Acetate or for further information write to your nearest Union Carbide India Office.

UNION CARBIDE

INDUSTRIAL PRODUCTS SALES DIVISION

UNION CARBIDE INDIA LIMITED

BOMBAY • CALCUTTA • DELHI • MADRAS

IWTUC 246A

HINDUSTAN ANTIBIOTICS

Bulletin

Vol. 4 **November 1961** **No. 2**

CONTENTS

EDITORIAL & ADVERTISEMENT OFFICES

Hindustan Antibiotics Ltd., Pimpri, Poona Dist.

INDIA

Hindustan Antibiotics Bulletin **is published on the 15th of February, May, August & November, and contains articles and reviews on all aspects of antibiotics production and use.**

The views expressed in this journal are those of the authors and do not necessarily represent those of the Company or of the editors.

Annual subscription : Rs. 6.00 (inland), 16 sh. or $ 4.00 (foreign). Single copy : Rs. 2.00 (inland), 4sh. 6d. or $1.25 (foreign).

ADVERTISERS INDEX

ANTIBIOTICS IN ANIMAL NUTRITION

AMONG the non-medical uses of antibiotics, their use in animal feeds has gained considerable importance. Large scale fermentation, particularly of the tetracyclines, is being done with the sole purpose of producing animal feeds. Poultry grow twice as fast to-day than they did twenty years ago. This is not only due to improved and planned breeding techniques, but also due to effective use of antibiotics in poultry feeding. Along with antibiotics, the mycelia and other extraction residues from the manufacture of antibiotics are also useful as animal feeds. The mycelium of *Streptomyces aureofaciens* from chlortetracycline fermentation, and that of *Penicillium chrysogenum* are extensively used in poultry feeds. One of the poultry feed formulations recommended by Dr. E. N. Moore of the Technical Co-operation Mission in India, contains 5 per cent dry mycelium from penicillin fermentation. The growth of chicks on the formulation in a 5-week period is reported to be more than 50 per cent over the controls. The more important antibiotics used in commercial feed stuffs are chlortetracycline, oxytetracycline and procaine penicillin. Streptomycin, bacitracin, and occasionally erythromycin, neomycin, gramicidin are also used in conjunction with one of the above three antibiotics. Inactivated antibiotics and non-antibiotic microbial products are also known in some cases to give growth responses in animals. Oral feeding is the method of choice. Subcutaneous administration has failed to give beneficial results in many cases. Ruminants respond more readily to antibiotic feeding than those with simple stomach.

There has been a continuous trend to increase the antibiotic level in the feed. As much as 300 p. p. m. of antibiotics are sometimes used. This high level feeding not only stimulates the growth of the animal but results in the deposition of the antibiotic in various tissues. This deposition is considered useful in the preservation of the animal if it is sacrificed for meat. Most of the antibiotics being unstable in moist conditions, insoluble salts such as procaine penicillin, calcium complexes of chlortetracycline and oxytetracycline are mixed with the feed, and then marketed.

Growth responses in chicks and hogs to antibiotic feeding are remarkable, but the exact reason for this phenomenon is not completely understood. Antibiotics are found to increase appetite for food and water. The requirement of vitamins is reduced, and antibiotics such as chlortetracycline corrects deficiencies due to folic acid or vitamin B_{12} in animals. Growth responses are more marked in animals kept under stress and poor environmental conditions. This gives the impression that antibiotics may control subclinical infections and thus promote growth. Several investigations point to the changes in the intestinal microflora on antibiotic feeding and lead us to assume that antibiotics may suppress the growth of feeders on important nutrients in

the intestines and thus make them available to the host. Increase in population of beneficial microorganisms is often considered to be one of the chief effects of antibiotic feeding. However, when the results of feeding antibiotics to germ-free animals are taken into account the above findings need to be modified. Antibiotics stimulate the growth in germ-free animals thereby indicating that their effect may be direct and not indirect by regulating the growth of intestinal microflora. The mechanism of action may be different in each case, and results of further studies are keenly awaited.

INTERNATIONAL SEMINAR ON ARTERIOSCLEROSIS

Bombay, 3rd, 4th and 5th February, 1962

Papers are invited for the above Seminar which will cover all aspects of arteriosclerosis. Special Sessions will be devoted to the following subjects :

1. Cardiovascular Arteriosclerosis
2. Neurological Arteriosclerosis
3. Peripheral Vascular Arteriosclerosis
4. Experimental Arteriosclerosis
5. Panel Discussion on Arteriosclerosis, which will be of special interest to general physicians.

It may be possible to arrange for return air/first class rail fares for those whose papers are selected for presentation at the Seminar. In addition, they will stay in Bombay for the duration of the Seminar as guests of the Organising Committee. The last date for receiving summaries is December 1st 1961. They should be sent to :—

Jt. Organising Secretaries,
International Seminar on Arteriosclerosis,
K. E. M. Hospital, Bombay-12.

The last date for registration of delegates is December 1st, 1961. There is no registration fee, but early registration is advisable as otherwise it may be difficult for the Organising Committee to arrange for accommodation. As international celebrities are expected, the Seminar will be widely attended.

PUBLIC RELATIONS IN THE STATE ENTERPRISES

IN recent years, particularly after the commencement of the Five Year Plans, the Union and State Governments in India are accepting wider responsibilities in the field of industrial development of the country. By the beginning of the current year twenty-two industrial undertakings were completed, nine were in various stages of completion, and there were twenty-three other commercial, financial, general development and miscellaneous corporations. In the ultimate analysis, the success of these state enterprises would be judged by the service they render to the people in terms of the quality and quantity of the right product made available at reasonable prices. The conduct of the enterprises would also receive increasing attention as employers of a large working force, and in regard to their vital role in the economic life of the country.

At the present stage of economic development, the public in India has no clear cut ideas about the performance or norms of conduct expected of state undertakings. Nevertheless, with growing literacy and political consciousness, there is an increasing awareness of and demand for, factual and up-to-date information about the state enterprises. This is but natural, and in most cases the public is also the consumer of the products turned out by these industries. The Indian press is also taking a growing interest in the governments' ventures in industry and there is need for an efficient organization in the state enterprises to feed the press with relevant information. Of course, in the case of industries associated with defence requirements full information may not be made public for security reasons. Again, policy making authorities are interested in knowing the needs of the public and its opinion of and attitudes towards, the services rendered by public sector enterprises. Public accountability is another factor to be taken into account in this context. The state enterprises publish annual reports which are presented to the Indian Parliament by the ministers concerned and questions may be raised about the working of these industries. The activities of the undertakings are also subject to review from time to time by the Estimates Committees appointed by the Parliament. The state enterprises have, therefore, to keep close contacts with public opinion, members of the legislature, and the press and provide a continuous flow of factual information about their working and achievements.

Most of the state undertakings were started after Independence and some of them are still in the initial stages of development. In a large measure these enterprises are basic or key industries turning out products to meet the needs of various government departments. At the present stage of their growth it is difficult to gauge the extent to which efforts have been made by them to assess public opinion. The existing public relations departments in some of the corporations do take note of public views and of discussions in the legislatures on the undertaking, through the local press. The departments maintain informal relations with newspaper representatives and with the technical trade press. Press conferences are called and hand outs used, most often when the annual report of the enterprise is to be released. Apart from annual reports, the companies also issue booklets and pamphlets to tell the story of the industry, its problems and development schemes. Some enterprises such as

Hindustan Antibiotics and the Hindustan Machine Tools, publish technical journals incorporating the results of research carried out by the staff members. House magazines of the informational type are also issued by a number of the companies. They also participate in commercial and technical exhibitions, and some maintain close contacts with the universities, learned bodies and research institutions.

All these public relations activities are quite desirable, but have limited value as techniques for educating and assessing public opinion. The effectiveness of the public relations programme in providing a convincing and factual picture of the undertaking and its role in the economic reconstruction of the country, very largely depends on the personnel in charge of such programmes. It is, perhaps, needless to add that a good record of progress and activities of an undertaking is a stimulant to successful public relations. Some may argue that a public relations department as a part of management is not necessary in a public sector undertaking. This reasoning is valid only if public relations is taken as synonymous to propaganda. That this is not the case is evident from the nature of the functions of public relations expected of a public sector undertaking.

There is an urgent need for realising the importance and contents of public relations activities in state enterprises, and the necessity to staff the public relations departments with adequately qualified and trained personnel. Standards of qualifications and training for such personnel must be drawn up and adequate provision made for imparting such training at the highest level. Such an integrated programme alone will lead to public relations departments which can effectively bring about a better understanding on the part of the public of the problems, achievements and role of state enterprises in particular, and of the industry in general, in the economic and social life of the nation.

—V. M. DHEKNEY

REVIEW

The Pharmacology of Tetracyclines

P. N. KAUL

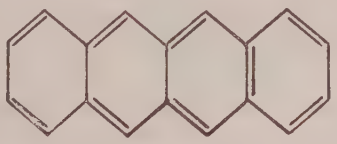
Hindustan Antibiotics Ltd., Pimpri, Near Poona

TETRACYCLINES are a group of broad-spectrum antibiotics obtained as metabolic products of various *Streptomyces* species. These drugs have attained considerable therapeutic importance in the management of specific and non-specific microbial infections. Their antimicrobial spectrum includes most gram positive and gram negative organisms, rickettsiae, lymphogranuloma venereum and psittacosis viruses, and several other non-specific micro-organisms. Non-therapeutically, they have found extensive use as animal feed supplements and in food preservation. Interest in the tetracyclines has been further enhanced by the recent addition of semisynthetically produced therapeutically promising agents to the group. With the increasing importance of these antibiotics in medicine, it is essential to know their pharmacological effects on and biological disposition in, the animal body. The knowledge of how these antibiotics are handled by the body may also help in designing new and therapeutically better tetracyclines, as has happened in the case of sulphonamides, Daraprim, etc.

It is the purpose of this paper to review the status of present knowledge of the pharmacology of therapeutically important tetracyclines, with particular reference to their absorption, distribution, metabolism and excretion.

CHEMISTRY AND PHYSICO-CHEMICAL PROPERTIES

Tetracyclines are chemically related compounds possessing a common nucleus (I) of substituted perhydro-naphthacene (Fig. 1). Tetracycline (Achromycin), which



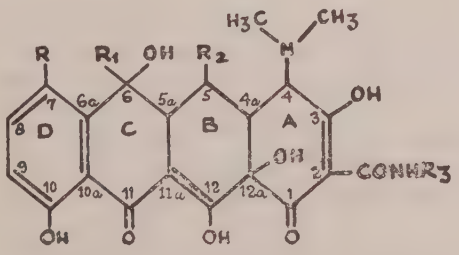


Fig. 1. STRUCTURAL FORMULAE OF TETRACYCLINES

I,	R_1	R_2	R_3	R_3
II,	H	CH_3	H	H
III,	Cl	CH_3	H	H
IV,	H	CH_3	OH	H
V,	Cl	H	H	H
VI,	H	CH_3	H	CH_2-N<□

may be considered as the parent compound of the family, is 4-dimethylamino-1, 4, 4a, 5, 5a, 6, 11, 12a-octahydro-3, 6, 10, 12, 12a-pentahydroxy-6-methyl-1, 11-dioxo-2-naphthacenecarboxamide (II). Chlortetracycline (Aureomycin) is the 7-chloro- (III) and oxytetracycline (Terramycin) the 5-hydroxy- (IV) analogue of tetracycline. McCormick *et al.*[55] isolated a group of 6-demethylated tetracyclines from the metabolic products of a mutant strain of *Streptomyces aureofaciens*, the organism that biosynthesizes chlortetracycline. Of these, 6-demethyl-7-chlortetracycline (Declomycin) (V) has achieved clinical prominence. In 1958 Siedel *et al.*[72] reported the synthesis and properties of yet another of the broad spectrum antibiotics, the N-(pyrrolidinomethyl) tetracycline (VI), available in the U.S.A. as Syntetrin and in Germany as Reverin. It is prepared from tetracycline by substituting a pyrrolidinomethyl group for one of the two hydrogens on the amide nitrogen of the parent compound. This compound is about 2,500 times more soluble than tetracycline, and has shown good promise for parenteral use. In Table I are summarized the physicochemical properties of these tetracyclines.

There are a number of other analogues obtained by chemical modification of the rings A-D of tetracycline. Some of these have even been isolated from various mutants of *Streptomyces* species. The chemistry and *in vitro* activity of these compounds have been reviewed.[11]

GENERAL PHARMACOLOGICAL EFFECTS

The main pharmacological effects produced by tetracyclines are on the gastrointestinal tract and mucous membrane ; and these appear to be due to local irritation. Tetracyclines being acidic substances, with at least three pk_a values [38,76] ranging from 3.3 to 9.7, their irritant property is likely due to the acidity. This is further substantiated by the fact that intravenous injections do not produce any gastrointestinal or mucous membrane disturbances. The commonly encountered symptoms include epigastric distress, anorexia, nausea and vomiting, oropharyngeal complications such as angular stomatitis, atrophic mucous membrane complications such as vaginitis, vulvar irritation and anal fissuring. All these symptoms disappear upon withdrawal of the oral tetracycline. The disturbances of the gastro-enteric canal may also be caused by superinfection by tetracycline resistant micrococci, *Candida albicans* and Proteus, and also by alteration in the microflora normally inhabiting the intestines. In general, tetracycline is believed to show somewhat less effects of this nature than its chloro- and oxy-analogues.[31]

Table II summarizes the LD_{50} data on various tetracyclines. It is interesting to note that although demethylchlortetracycline (DMCT) has been put to extensive laboratory and clinical studies, no data on its acute toxicity is available. Ther and Vogel,[79] while studying the toxicity of N-(pyrrolidinomethyl) tetracycline (PMT) on various animal species, found no correlation between the dosage and body weight. Mice were found to be more susceptible to PMT preparations.

Mice tolerate daily oral doses of 100 mg/kg of chlortetracycline for 12 weeks, and of 75-200 mg/kg of oxytetracycline for 6-8 weeks, whereas rats tolerate daily oral amounts of 100-400 mg/kg of tetracycline for 30 days, of 100-200 mg/kg of chlortetracycline for 12 weeks, and of 75-200 mg/kg of oxytetracycline for 6-8 weeks.[74] In dogs, oral doses of 200-250 mg/kg per day of tetracycline for 12-14 weeks, of 100-200 mg/kg per day of chlortetracycline for 12 weeks and of 75-200 mg/kg per day of oxytetracycline for 6-8 weeks are nontoxic.[74]

TABLE I : PHYSICO-CHEMICAL PROPERTIES OF TETRACYCLINES

Properties	Tetracycline HCl	Oxytetracycline HCl	Chlortetracycline HCl	Demethylchlortetracycline sesquihydrate	N-(pyrrolidinomethyl)-tetracycline
Nature	Amphoteric (as base)	Amphoteric (as base)	Amphoteric (as base)	Amphoteric (as base)	Amphoteric
Mol. Formula	$C_{22}H_{24}N_2O_8HCl$	$C_{22}H_{24}N_2O_9HCl$	$C_{22}H_{23}ClN_2O_8HCl$	$C_{21}H_{21}ClN_2O_8$-1.5H_2O	$C_{27}H_{33}N_3O_8$
Mol. Wt.	480.5	496.5	515.36	491.88	527.3
Colour and cryst. form	Orthorhombic, yellow	Yellow platelets	Vitreous yellow, rhomboid	—	Fine pale yellow needles
M. p.	214° d.	190-94° d.	above 210° d.	174-78° d.	162-65° d.
$[\alpha]_D^{25°}$	-257.9°(0.1*N* HCl)	-196.6 (0.1*N* HCl)	-240° (H_2O)	-258° (0.1*N* H_2SO_4)	—
λ_{max} (mμ)	220, 268, 335 (0.1*N* HCl)	249, 276, 353 (pH 4 buffer)	230, 262, 367 (0.1*N* HCl)	Same as that of chlortetracycline	—
pk_a values	3.30, 7.68, 9.69*	3.27, 7.32, 9.11*	3.30, 7.44, 927*	Likely to be the same as chlortetracycline	1st. pk_a likely to be smaller; 2nd and 3rd nearly the same as for tetracycline. Isoelectric point, 7.9.
Solubility, mg/ml. at 28°	H_2O, 10.9; MeOH, >20 ; EtOH, 7.9 ; C_6H_6, 2.5 ; EtAc, 0.75; $CHCl_3$, 2.85; Et_2O, 0.60.	H_2O, 10^3; EtOH, 33; Abs. alc., 12; C_6H_6, 0.09; $CHCl_3$, 0.40	H_2O, 8.6 ; MeOH, 17.4; EtOH, 1.7 ; Et_2O, 0.085 ; C_6H_6, 0.09; EtAc, 0.35.	Likely to be more soluble in H_2O than chlortetracycline.	2,500 times more H_2O soluble than tetracycline ; soluble in EtOH, dil. acids and alkali.
Stability	Stable at pH 7 ; 50% loss in 12 hr. at pH 8.85 ; 40% loss after 8 days at pH 1.	Stable at 100° for 4 days ; stable at pH 1-2.5 at 25° for at least 30 days.	Stable at pH 2.5 ; unstable at pH 7 and above ; ther-; molabile in acids and bases.	Thermostable in acids and bases.	Same thermostability as tetracycline ; stable at pH 5 for several hr.

Cf. Albert, *Nature* **172,** 201 (1953).

TABLE II

LD_{50} OF TETRACYCLINES IN VARIOUS ANIMAL SPECIES

The Antibiotic (HCl)	LD_{50} mg/kg (19/20 confidence limit) in			References
	Mice	Rats	Other species and comments	
Tetracycline	162 (i.v) 190 (i. p) 2,130 (oral)	129 (i. v) 321 (i. p) >1,500 (oral)	Dogs tolerated repeated doses of 100 mg/kg orally	19
Oxytetracycline	178 (i.v) 830 (s. c) 6,693 (oral)	260 (i. v)	Rabbits approx. 75 (i.v). Dogs approx. 150 (i. v)	61
Chlortetracycline	155 (i. v) 192 (i. p) >3,000 (oral)	160 (i. v) 325 (i. p) >3,000 (oral)	Dogs, rabbits and guinea pigs tolerated i. v. and oral doses of 50 mg/kg	14, 34
PMT	75 (i. v)		Rabbits — 45 Dogs — 95	79

In man, daily oral doses in excess of 500 mg of the earlier established agents and DMCT are known to produce nausea, vomiting and diarrhoea, but smaller doses are well tolerated. Although these effects are believed to be less frequent with tetracycline than with its chloro- and oxy-analogues[22], DMCT produces much less gastrointestinal symptoms than even tetracycline.[16,88].

Effect on cardiovascular system

Tetracycline administered intravenously to dogs under anaesthesia in doses upto 100 mg/kg produces no significant effects on blood pressure. It has no effect on blood clotting time in rats.[16] A 100-200 mg/kg daily oral dose of chlortetracycline for 12 weeks produces no ill-effects on haematology, clotting time, blood sugar and blood pressure in dogs.[34] There is no detailed report on the effects of oxytetracycline on the cardiovascular system. In a study of the effect of oral tetracyclines on the coagulation of rabbit blood, Zolutukhin[88] found that chlortetracycline reduces the coagulation time as well as the time of plasma recalcification. At the same time, tolerance of plasma to heparin is increased, indicating an antiheparin effect. Neither tetracycline nor oxytetracycline show any such effect.

Table III gives the blood picture of mice following administration of various tetracyclines.[14] It appears from the table that the tetracyclines induce no significant blood dyscrasias. However, with oxytetracycline a rare occurrence of the phenomenon has been reported.[83] Tetracycline in doses of 30 and 60 mg/kg and chlortetracycline in a dose of 30 mg/kg have been found to cause hyper-ferremia in rabbits lasting about 24 hr.[12] In low doses, however, chlortetracycline causes only a transient hyper-ferremia. Demethylchlortetracycline has been found to increase the number of circulating eosinophils.[57]

Effect on the liver

Tetracyclines cause degenerative changes in hepatic cells demonstrable by histological examination. A quantitative study has revealed that chlortetracycline produces

TABLE III

BLOOD PICTURE OF MICE FOLLOWING MULTIPLE ORAL DOSES OF TETRACYCLINES

Antibiotic	Dose mg/kg	Number of			Complete Blood Count						
		Mice	Doses	Days	Hb g/100 ml.	R.B.C. Millions per c. mm.	W.B.C. Thousands per c. mm.	% Differential			
								Neut.	Eos.	Lymph.	Mono.
None	H_2O	10	31	43	13.3	9.4	16.6	19.9	1.9	78.0	0.5
Tetracycline	100	10	30	42	14.3	10.7	22.8	27.6	1.7	70.2	0.5
Chlortetracycline	100	10	30	42	13.6	9.7	16.5	12.2	1.7	85.7	0.4
Oxytetracycline	100	10	31	43	12.9	9.5	22.6	23.6	2.5	73.8	0.1

the most consistent deposition of fat in rat liver cells, whereas oxytetracycline produces a less and tetracycline the least such effect. However, all the three agents show a significant lipid deposition. The effect due to chlortetracycline is not reversed by liver extract, vitamin B_{12} or various other vitamins, but is spontaneously reversed at the end of 72 hr. following tetracycline injection.[71]

From several reports, Lepper[50] has concluded that chlortetracycline in some cases may actually protect the liver from disease caused by endogenous bacterial toxins. However, this is possible only in smaller doses and a prolonged use of higher doses usually results in a clinically damaged liver. The protection afforded by chlortetracycline against liver damage due to bacterial toxin or to substances produced by intestinal flora (*e.g.*, liberation of phenol from tyrosine, [68] or of trimethylamine from choline[40]) appears to be due to the drug-induced favourable changes in the flora. Florey[28] has reviewed the clinical hepatotoxicity caused by chlortetracycline, and it appears that the intravenously given drug is quite harmful if used over a prolonged period. No data are available on liver damage due to DMCT and PMT, but these agents are also likely to show similar effects on the liver.

The mechanism of hepatic degeneration caused by tetracyclines is not known. It is possible that these antibiotics arrest, through chelation, the magnesium ions that are essential for the energy requiring processes of lipid metabolism. This can result in an incomplete catabolism and therefore a local deposition of fat in the liver cells. The role of halogen in the liver damage cannot be ruled out, for, of all the tetracyclines, chlortetracycline causes maximum degeneration. However, it would be interesting and also confirmatory of this fact to quantitatively study the fatty degeneration of the liver caused by newly isolated "halometabolites"[62] that are biosynthesized by various mutants of *Streptomyces aureofaciens*.

Effect on the kidney

Renal complications induced by various antimicrobial agents have been reviewed by Finland and Weinstein.[24] Tetracyclines in general are believed to have no harmful effect on the urinary tract. However, oxytetracycline in patients with impaired renal function may cause azotemia due to increased general tissue catabolism, and high serum levels may result

from failure to excrete the drug.[3] Farhat *et al.*[21] have reported that reduced renal excretion in patients with renal disease leads to accumulation of the antibiotics in the serum to toxic levels. Serum levels of 20 μg/ml initiates symptoms of toxicity as well as the elevation of non-protein nitrogen. Levels of 80-320 μg/ml are found to be lethal. There is a close correlation between the levels of non-protein nitrogen and the serum levels of tetracycline and of oxytetracycline. Chlortetracycline has been found to cause a mild diuresis[34] to the extent of one third the activity of caffeine. Dowling *et al.*[17] have also found an increase in the rate of urine flow with the antibiotic.

Effect on enzymes

Most enzymatic processes in the body require trace amounts of certain metal ions for their normal functioning. The inhibitory effect of tetracyclines on enzymes is most likely due to their ability to chelate these essential ions. Thus the antibiotics are reported to inhibit[82] and uncouple[10] oxidative phosphorylation in mitochondria. This is perhaps due to the binding, by the tetracyclines, of M_g^{++} ions essential for oxidative processes. Recently phase-contrast and fluorescence microscopy have revealed a selective localization of tetracyclines in the mitochondria of living cells of tissue cultures and fresh homogenates.[18]

Tetracycline and its chloro- and oxy-analogues inhibit fatty acid oxidation[10] and arginine catabolism.[39] Malek *et al.*[53] have found that tetracyclines inhibit the activity of pancreatic α-amylase and lipase, and have claimed this inhibition to be of clinical significance. The inhibition is increased in presence of Ca and Mg, and nearly reversed by addition of citric acid. They have suggested that the gastrointestinal disturbances due to tetracyclines may be a result of dyspepsia and related imbalance of digestive processes caused by the inhibition of the pancreatic enzymes. However, this may only be an additional likely cause rather than the sole cause of the gastrointestinal complaints following tetracycline therapy.

While some enzymes are inhibited by tetracyclines, others inhibited by heavy metals may actually be reactivated by the addition of these agents which remove such inhibitory metals through chelation. Thus, the berylium-induced inhibition of rat-plasma alkaline phosphatase is 75 per cent reversed by the addition of oxytetracycline or other suitable ligands.[51]

Tetracycline, chlortetracycline and oxytetracycline have been found to cause a decrease in the liver deaminase, accompanied by increased excretion of total nitrogen, predominantly of amino origin.[65]

Other effects of interest

There have been instances of dermatitis medicamentosa, especially maculopapular and scarlatiniform rashes and photosensitivity with chlortetracycline therapy.[9] Phototoxicity due to DMCT has drawn considerable attention during the past two years. Several cases have been reported, [20, 57, 70] where exposure to natural or artificial source of ultraviolet light resulted in dermatological reactions of varying sort in patients receiving the antibiotic, the most common of the reactions being erythematous rash. Trams *et al.*[80] reported that an intravenous dose of 50-100 mg/kg of chlortetracycline increases the weight of the adrenal gland in rats and stimulates cortical activity. Moreover, adrenalectomized rats tolerate less of the drug. Tetracycline has been found to potentiate the curarizing effect of *d*-tubocurarine.[4]

ABSORPTION AND BLOOD LEVELS

A large number of reports have appeared on the post-absorptive serum levels of tetracyclines in animals and in humans.

There being no precise assay method of desired high sensitivity available, practically all these levels are based on microbiological assays which require a 24-hr. incubation of the antibiotic with some micro-organism. Besides inaccuracies and errors inherent in the bioassays, the major problem in determining the exact concentration of a tetracycline in a particular sample at a certain fixed time, has been the deterioration with time of these antibiotics. Therefore, all quantitative data on absorption and blood levels should be considered only for comparative purposes and not as absolute values. The data, nonetheless, has proved of value in clinical evaluation of tetracyclines and has also helped the physician in his discretion while prescribing these agents. Information regarding absorption and blood levels of tetracycline and of its chlor- and oxy-analogues in animals has been reviewed.[16, 50, 58, 81]

The relative merits of various routes of administration to humans of tetracycline and its chloro- and oxy-derivatives have been extensively discussed by Florey.[28] From a critical study of the levels of these antibiotics, it appears that similar serum levels are obtained by 8-hourly oral ingestion and by intravenous administration of tetracycline and oxytetracycline, whereas chlortetracycline shows lower levels by oral route. In all cases, however, the intramuscular injections produce significantly lower levels than the other two routes. By adding hyaluranidase to the antibiotic prior to intramuscular administration, high serum levels, comparable to post-intravenous levels, have been obtained.[77] Other routes of absorption *e.g.* subcutaneous, vaginal, rectal etc., also result in good therapeutic serum levels. However the problem of local irritation limits the practical value of these routes.

DMCT after single or repeated oral dose is absorbed to a slightly less extent than tetracycline, but the antibacterial activity attained in the serum is much greater and better sustained than any of the three established tetracyclines.[36, 46] The prolonged level results, possibly, from the slower renal clearance of the agent. On the basis of the given dose, DMCT in children shows an absorption comparable to that of tetracycline, but the serum antibacterial activity with the former is about three times that of the latter.[69] Single oral 250-mg dose gives equally high but more prolonged serum concentration than does chlortetracycline. On continued daily administration of DMCT for 5 days, there is a slight but definite accumulation of it in the serum due to slower rate of its renal excretion.[5]

N-(pyrrolidinomethyl) tetracycline (PMT) has been under clinical trials in Germany and in the United States for over 2 years now. It is given as deep intramuscular or intravenous preparation. Although it does not produce much local irritation at the site of injection,[48, 81] the commercial preparation contains added local anaesthetic. Intramuscularly given PMT is absorbed more than 70 per cent better than tetracycline or its phosphate complex.[41] Wagner *et al.*[84] have found that oral absorption is not effective enough to produce serum levels obtained from intravenous injection, and that the latter route produces therapeutically effective levels at even 12 hr. after administration. Cronk *et al.*[13] in a detailed study on absorption and excretion of PMT report that the average serum concentration is proportional to the amount of the antibiotic injected. There was no evidence of cellulitis at the site of injection as is commonly seen following the injection of tetracycline preparations.

The average serum levels of antibacterial activity, calculated as tetracycline, in the blood of humans after single 500-mg. oral doses of the four tetracyclines have been studied by Finland and coworkers.[25] A similar study on intramuscular PMT has been made by Cronk *et al.*[13]

Enhancement of absorption

Some manufacturers have designed several tetracycline formulations with added adjuvants, and have claimed that adjuvants such as glucosamine, citric acid etc., enhance the absorption of tetracycline. The reported differences in the relative blood levels of these formulations have been primarily due to lack of properly controlled experimentation and to variation errors inherent in the analytical procedures employed for estimating the blood levels of tetracycline. The controversial issue of enhanced absorption of tetracycline has been discussed [26, 47] and editorially reviewed.[59] That, in fact, the added agents do not significantly affect the absorption of tetracycline, is further supported by the most recent findings of O'Reilly and Nelson.[60] These authors studied the absorption of tetracycline hydrochloride, tetracycline phosphate complex, tetracycline hydrochloride with added glucosamine hydrochloride and tetracycline hydrochloride with added citric acid, by excretion rate measurements and found that tetracycline absorption was essentially the same for all preparations.

Fundamentally, there is no reason why the adjuvants should enhance the absorption of tetracycline, unless the absorption is an enzymatic process and the adjuvants inhibit or stimulate the enzymes involved ; or the adjuvants have inactivating effect on certain metal ions in the gastrointestinal tract which normally bind the tetracycline and thereby reduce its absorption. Tetracycline has been reported to be absorbed passively,[63] and as such it is difficult to visualize why the added substances should enhance its absorption. Although it is known that tetracycline chelates calcium and magnesium, and that the presence of these metals depresses the absorption of the antibiotic, administration of ethylenediaminetetraacetic acid (EDTA) has no marked influence on its absorption.[6] The stability constant of the calcium chelate of EDTA is much greater than the corresponding chelate with, for example, citric acid, one of the adjuvants used as an absorption enhancement factor.

The tetracycline hydrochloride available commercially prior to October 1957 contained added dicalcium phosphate, and it is possible that the controversial claims of enhanced absorption due to various adjuvants resulted from a comparison of the blood levels produced by these preparations with the levels produced by the adjuvant-added formulations. Calcium diphosphate present in the earlier preparations would naturally depress the absorption of tetracycline.

The observations[46] on DMCT are interesting in this connection. This agent has been found to produce much higher and prolonged levels of antibacterial activity in the serum than tetracycline after single or multiple doses. Its half-life in the serum is 44 per cent longer than that of tetracycline, suggesting that smaller and less frequent doses of DMCT might be equally effective as the conventional doses of tetracycline.

DISTRIBUTION

Distribution of tetracycline and its oxy- and chloro-analogues has been reviewed in detail by several authors [16, 28, 50, 58]. In general all these agents get fairly rapidly distributed into the fluids and tissues of the body. Welch[85] has found that at 4 hr. after single oral dose of 0.5 mg/kg in rabbits, the concentration of oxytetracycline in the brain, the skin and the heart is much greater than that of chlortetracycline, and also several times its own blood concentration. Chlortetracycline is found in greater concentration than oxytetracycline in the liver, the kidney and the lungs. Katsura and Aoki,[42] while studying the visceral distribution of tetracycline in mice, have found that the concentration in the various organs in order of decrease are liver, large intestines, small

intestines, kidneys, lungs, spleen, heart blood, brain. Similar studies with chlortetracycline[2] have revealed nearly the same pattern of distribution with the highest concentration in the liver occurring at 5 hr. after oral administration, and at 2 hr. after subcutaneous injection. An interesting observation was that a considerable amount of the antibiotic was found in the intestines even after subcutaneous administration. This clearly suggests that chlortetracycline is excreted via the bile into the gut. The explanation of Aoki[2] that the drug is excreted through the intestinal wall into the lumen appears less likely possible. Studies on oxytetracycline have revealed that, after an intramuscular depot injection to mice, the antibiotic is rapidly reabsorbed and accumulated in the reticulo-endothelial system, with high levels in the liver and in the kidney through which it is excreted.[35]

Following an intravenous injection of various tetracycline analogues in normal young men, an initial rapid decline of serum concentration, representing distribution in various body fluids and tissues, has been observed.[48] The half-life of active drugs in serum, as calculated, appears to be the longest for DMCT (12.7 hr.) and the shortest for chlortetracycline (5.6 hr.), whereas oxytetracycline and tetracycline have intermediate values (9.2 hr. and 8.5 hr. respectively). The relative volumes of distribution of the four analogues are 1.59 for tetracycline, 1.89 for oxytetracycline, 1.48 for chlortetracycline and 1.79 for DMCT. Plasma protein binding in humans as determined by equilibrium dialysis, shows a mean percentage of the bound drugs as 24 for tetracycline, 20 for oxytetracycline, 48 for chlortetracycline and 41 for DMCT. Studies with radio-labelled tetracycline analogues have revealed these values in human serum as 31 ± 6 for tetracycline, 64 ± 4 for chlortetracycline and 51 ± 2 for DMCT.

Knothe[45] found that a 20 mg/kg oral dose of DMCT in rabbits and dogs shows a distribution similar to that of tetracycline, but relatively higher and longer lasting levels of the former occur in the serum and organs. With repeated dosage, cumulative effect, as reflected by persisting higher tissue levels, has been observed with both the antibiotics. The tetracyclines localize in bone and tumour tissue of tumour bearing animals, as evidenced by a fluorescence exhibited over long periods by these tissues. The fluorescence has been shown to be due to the intact tetracycline molecule, complexed reversibly to a peptide which is one of the normal constituents of the mouse sarcoma tissue.[52].

BIOTRANSFORMATION

Tetracyclines possess several chemically active sites and it is possible that these agents are biotransformed (metabolized) in the body to several metabolites. Of course, part of the given dose of each agent is excreted unchanged in urine and in feces, as claimed on the basis of microbiological assay. Whether the antibacterial activity in these excreted materials can be solely attributed to the unchanged drug, or that what is assayed also includes some active metabolites, has not yet been established. However, according to a recent report[43] on the metabolism of tetracycline-H-3 and randomly labelled tetracycline-C-14 given intraperitoneally and orally to rats, approximately 90 per cent of the activity has been found in urine and feces. A significant proportion of the remainder remains bound on the skeleton of the animal as chelated tetracycline. The labelled tetracycline excreted after administration to rat and dog has been claimed to be chemically unchanged. Williams[86] suggests that a part of these antibiotics is inactivated in the body. The lack of information regarding the biotransformation of tetracyclines is possibly due, firstly, to the use of nonspecific assay methods, and secondly, to the fact that no systematic attempt has been made to study the metabolism of these antibiotics in the body.

EXCRETION

The main routes of elimination of tetracyclines from the body are urine and feces, though the antibiotics find access to all the other body fluids as well. Most excretion studies have been done on humans.

Tetracycline is excreted in urine over 24 hr. to an extent of 20 to 60 per cent after a single oral dose of 0.5 g.,[54] to that of 15 to 27 per cent after single dose of 1 g.,[66] and to 20 to 55 per cent after 1.4 g.[56] Urinary excretion of tetracycline in traces appears to continue even to the ninth day after oral administration of the drug.[87] In feces about 2.2 mg. tetracycline per g. of wet stool is excreted after a total 2-g/day oral dose, and up to 18 μg/g of wet stool at 24 hr. after a single intravenous dose of 0.5 g.[54] Posner *et al.*[64] have found that when mothers are given 0.5 g. of tetracycline four times a day, the concentration in their milk is usually half the serum concentration. Tetracycline level in the prostatic fluid of humans collected one hour after an intravenous dose of 0.5 g. of the antibiotic is from 0.16 to 0.25 μg/ ml[8]. Recent studies[60] on the absorption of various tetracycline preparations by excretion rate determination have revealed that about 28±5 per cent of the given oral dose (200 mg.) is excreted as tetracycline (calculated as hydrochloride) during the first 8 hr. after administration.

Oxytetracycline is excreted in urine over 24 hr. to the extent of 100-200 mg. after a single oral dose of 0.5 to 2 g.,[85] and to that of 9-30 per cent of the given dose.[66] The randomly C-14 labelled antibiotic appears to be excreted in the urine to a significantly less degree in subjects with nephrotic syndrome than in subjects with liver damage.[49 85] In the feces, it is excreted up to an extent of 1 mg/g of wet stool after a single dose of 2 g.[85]

TABLE IV

EXCRETION DATA ON FOUR TETRACYCLINES

Parameters	Tetracycline	Oxytetracycline	Chlortetracycline	Demethylchlortetracycline
t ½ in serum	8.5	9.2	5.6	12.7
RDV (% body wt.)	159.0	189.0	148.0	179.0
% dose excreted in 96 hr.	60.0	70.0	18.0	39.0
Renal excretion (%/hr.)	4.9	4.4	2.9	2.1
Time required for 50% renal excretion (hr.)	14.1	16.0	25.9	35.5
Non-renal excretion (%/hr.)	3.3	3.2	9.5	3.4
Time required for 50% non-renal excretion (hr.)	21.7	24.0	7.3	20.1

Chlortetracycline is excreted in urine over 24 hr. to the extent of 11 to 25 per cent of the given oral dose.[66] Renal clearance studies[73] have indicated that the antibiotic is cleared at about 3.5 per cent of the glomerular filtration rate, and approximately one-eighth of a dose appears in the urine over 24 hr. Maximum concentration up to 60 μg/ml. in the urine is usually found at 6-8 hr. after a single 1-g. oral dose.[73] Considerable amounts of the antibiotic to the

extent of 500 μg/g wet stool is found in the feces.[85]

Demethylchlortetracycline[48] is excreted in the urine of humans over 96 hr. to the extent of 42 per cent of the given oral dose. Its non-renal rate of disappearance is about 3 to 4 per cent per hour. The renal clearance is 27± 8 per cent of the simultaneous endogeneous creatinine clearance. Levels from 2 to 32 times the serum levels are found to concentrate in the bile.

Cumulative urinary and fecal excretion of DMCT in humans has been studied by Sweeny *et. al.*[78] The intravenously administered compound is excreted in the urine to an average extent of 52 per cent, and in the feces to that of 6 per cent during 104 hr. The route of excretion bears some relation to the serum level. Thus the subject with the highest serum concentration excretes about 43 per cent in the urine and 2.3 per cent in the feces, whereas the one with the lowest serum level excretes only 9 per cent in ihe urine and 72 per cent in the feces. Higher fecal concentrations after oral dosage support that the absorption through oral route is less complete.

N-(pyrrolidinomethyl) tetracycline (PMT) excretion in the urine after 250-mg. intravenous dose is 150-200 μg/ml in the first 6 hr., about 115 μg/ml for the next 6 hr., and more than 100 mg/ml for the 12-24 hr. sample.[15] Patients with normal renal function excrete in 24 hr. more than half of the intravenously given 250-mg. dose. Post-intramuscular urinary excretion with the same dose is only 40.1 per cent during 24 hr. Urinary excretion in humans over 30 hr. after a single intramuscular dose is 50-60 per cent regardless of the amount injected,[13] approximately 20 per cent being excreted in the first 6 hr. Fecal excretion of intravenous PMT in humans is considerably lower than that of oral tetracycline.[81] The excretion in rabbits after oral administration is about 6 to 10 times that after intravenously given PMT.

Lactating women, receiving a single intravenous 275-mg. dose of PMT, excrete the antibiotic in milk with a peak concentration at 4 hr. after the injection, though smaller concentrations averaging 0.32 μg/ml are present even after 24 hr.[32] Repeated administration on consecutive days does not show any cumulative effect on the concentration of the agent in the milk.

Kunin *et al.*[48] have reported a comparative excretion study of various tetracyclines. The renal clearance of each analogue, expressed as percentage of a simultaneous endogenous creatinine clearance, is tetracycline, 62±8 ; oxytetracycline, 85±14 ; chlortetracycline, 30±8 ; DMCT, 27+8. Table IV summarizes some of the data on excretion of these four tetracyclines.

CONCLUDING DISCUSSION

It is a common practice to claim better therapeutic efficacy of an agent on the basis of its higher concentration in the serum following administration of its normal doses. However, this evaluation may not be valid in the case of localized infections. In such cases it is the antibiotic concentration in the infected area rather than the blood levels that should really determine the therapeutic efficacy of the drug. Significance of tissue levels versus blood levels has been pointed out by Spitzy and Hitzenberger[75] who have developed a formula for calculating the "distribution volume" of several antibiotics including tetracyclines. According to these authors high blood levels result in low localized tissue levels and vice versa. The greater the distribution volume, the higher the concentration of the antibiotic in the tissues. Of the three established antibiotics, tetracycline has the highest distribution volume.

The calculation of distribution volume involves the administered dose according to the following formula :—

$$\text{Relative distribution volume (RDV)} = \frac{\text{dosage in mg}}{\propto (\text{mg/ml}) + \text{wt. in mg.}} = \frac{\text{ml}}{\text{g.}}$$

where *a* is the serum level at zero time after injection, assuming that the steady state equilibrium had developed instantly. Obviously, RDV does not mean much with drugs that are not completely absorbed after oral administration. One is apt to obtain false values with, for example, tetracyclines.

The phototoxicity due to tetracyclines, especially DMCT, has interesting implications. It possibly suggests that either the intact drug molecules or their biotransformation products may be distributed intracutaneously or in the subcutaneous areas. The development of any dermatological reactions on exposure to ultraviolet light may, then, be due to (i) a toxic compound synthesized from the distributed molecules by the energy of ultraviolet light or (ii) the damaging effect of ultraviolet light (region 230-360 mμ) itself when it penetrates the skin while being trapped by the subepithelially distributed tetracycline molecules which possess at least three absorption maxima in that harmful range of electromagnetic spectrum. It would be interesting to test the validity of either of these possibilities by a quantitative study of the epithelial and subepithelial distribution of tetracyclines, particularly DMCT. More frequent occurrence of phototoxicity with this member, than with any other tetracycline, suggests that the intact molecule rather than any metabolite may be responsible for the phenomenon just described, because being much more stable than chlortetracycline, DMCT is more likely to remain intact in the body at least till it gets distributed. Disappearance of the dermatological symptoms upon withdrawal of the drug supports that the drug molecules are necessary for the toxicity. The role of light energy is evidenced by the fact that the symptoms also disappear when the skin is protected from the ultraviolet light.

We know chelation reduces the toxicity of toxic ligands and toxic metals. There are instances, however, where the chelate complex may actually be even more toxic than either the ligand or the metal individually. Thus the tetracycline induced inhibition of pancreatic *a*-amylase and lipase is enhanced by the presence of calcium and magnesium ions.[53] Albert[1] has reviewed other cases where chelates in general are more toxic than the metal ion or the ligand separately. The increased toxicity may be a result of increased cell penetrating ability of the chelates through, perhaps, their enhanced lipid solubility.

The preferential localization of tetracyclines in bone and tumour tissues has initiated useful activity in cancer field. It has led to the synthesis of I-131-isostere of chlortetracycline and a study of its distribution in the body.[85] Preliminary results have revealed the potential of this labelled compound as a tumour detecting agent. Also, Klinger and Katz[44] have detected fluorescence in the gastric carcinoma tissue smear of humans following oral administration of tetracycline. In contrast, patients with diseases other than the cancer did not show any flourescence in the smear. It may be possible now to prepare carcinostatic compounds complexed reversibly with tetracyclines, such that, after localization in the tumour tissue, the anticancer part is liberated to produce its effect. The significance of such a development is obvious.

From literature reports, DMCT appears to be an oral tetracycline of choice. It is claimed to maintain higher antibacterial serum levels over prolonged periods, presumably, due to its longer half-life in serum and its lower renal clearance, and as such it may be administered at less frequent intervals, minimizing thereby the gastrointestinal complications. However, all these claims in favour of DMCT need a reappraisal. As has been emphasized earlier, the microbiological assays with *Bacillus cereus* as the test organism, on which the claimed higher antibacterial serum levels have been based, are not very specific

indicators of the therapeutic potency. For example, it has been noted that DMCT produces much lower antibacterial blood levels than tetracyline when assayed against streptococcus, a comparatively more common pathogen. Furthermore, it is not as well tolerated as is tetracycline. In the light of what has been said, the present status of DMCT as a better oral tetracycline is uncertain.[1a] Only further controlled experimentation and clinical trials can establish its future.

As a parenteral medication to combat acute tetracycline sensitive infections, intravenous PMT appears to be preferable. An interesting clinical study over six years has revealed that the drug may be used quite effectively in cases of pyelonephritis with apparently fixed hypertension.[7]

Studies on the relative merits of the four tetracyclines have revealed that oxytetracycline is the most effective antibiotic against *Pseudomonas pyocyanea*, whereas chlortetracycline shows greater activity against some of the gram-positive cocci.

Florey[28] has reviewed the comparative effectiveness of tetracyclines and bacitracin against amoebic dysentery and has concluded that oxytetracycline is the most effective agent.

Oxytetracycline formulation has been found to cause least amount of necrosis and tissue damage after intramuscular administration.[33] It would be interesting to carry out further work to establish this useful advantage of oxytetracycline over the other agents of the tetracycline group of antibiotics.

REFERENCES

1. Albert, A. Selective toxicity. 2d. ed. London, Methuen and Co., Ltd., 1960. Chapter 9

1a. *Antibiot. and Chemother.* **11,** 427 (1961)

2. Aoki, T. *Acta Med. Biol.* **7,** 1 (1959)

3. Bateman, J. V., *et al. Antibiot. Ann.* (1953-54) p. 531

4. Bezzi, G., and Gessa, G. L. *Nature* **184,** 905 (1959)

5. Boger, W. P., and Gavin, J. J. *Antibiot. Ann.* (1959-60) p. 388

6. Boger, W. P. *New Engl. J. Med.* **261,** 827 (1959)

7. Bohn, H., and Roch, E. *Ger. Med. Monthly* **5,** 8 (1960)

8. Borski, A. A., *et al. Antibiot. and Chemother.* **4,** 905 (1954)

9. Brainerd, H. D., *et al. ibid.* **1,** 447 (1951)

10. Brody, T. M., *et al. ibid.* **4,** 867 (1954)

11. Burstall, M. L. *Manuf. Chem.* **31,** 474 (1960)

12. Chernukh, A. M., and Balashova, V. A. *Antibiotics* **4,** 460 (1959)

13. Cronk, G. A., *et al. Am. J. Med. Sci.* **241,** 1 (1961)

14. Cunningham, R. W., *et al. Antibiot. Ann.* (1953-54) p. 63

15. Dimmling, T. *ibid.* (1959-60) p. 350.

16. Dowling, H. F. Tetracycline, New York. Med. Encycl. Inc., 1955

17. Dowling, H. F., *et al. Ann. N. Y. Acad. Sci.* **51,** 241 (1948).

18. Du Buy, H. G., and Showacre, J. L. *Science* **133,** 196 (1961)

19. English, A. R., *et al. Antibiot. Ann.* (1953-54) p. 70.

20. Falk, M. S., *J. Am. Med. Assoc.* **172,** 1156 (1960)

21. Farhat, S. M., *et al. Antibiot. Ann.* (1957-58) p. 426.

22. Finland, M., *et al. J. Am. Med. Assoc.* **154,** 561 (1954)

23. Finland, M., *et al. Antibiot. Ann.* (1959-60) p. 440.

24. Finland, M., and Weinstein, L. *New Engl. J. Med.* **248,** 220 (1953)

25. Finland, M., *et. al Antibiot. Ann.* (1959-60) p 375.

26. Finland, M. *Antibiot. and Chemother.* **5,** 359 (1958)

27. Finland, M., and Garrod, L. P. *Brit. Med. J.* **2,** 959 (1960)

28. Florey, M. E. Clinical applications of antibiotics. London, Oxford University Press, 1957, V. 2.

29. Frei, E., *et al. New. Engl. J. Med.* **252,** 173 (1955)

30. Garrod, L. P., and Waterworth, P. M. *Antibiot. Ann.* (1959-60) p. 440.

31. Goodman, L. S., and Gillman, A. The pharmacological basis of therapeutics. 2d. ed. New York, MacMillan Co., 1955, p. 1385.

32. Graf, H., and Riemann, S. *Deutsch. med. Wochen.* **84,** 1694 (1959); cit. *Nutr. Abst. Rev.* **30,** 2624 (1960)

33. Hanson, D. J. *Antibiot. and Chemother.* **11,** 390 (1961)

34. Harned, B. K., *et al. Ann. N. Y. Acad. Sci.* **51,** 182 (1948)

35. Helander, S., and Bottger, L. E. *Acta Med. Scand.* **147,** 71 (1953)

36. Hirsch, H. A., and Finland, M. *New Engl. J. Med.* **260,** 1099 (1959)

37. Hlavka, J. J., and Buyske, D. A. *Nature* **186,** 1064 (1960)

38. Hochstein, F. A., *et al. J. Am. Chem. Soc.* **75,** 5455 (1953)

39. Iyer, S. N., *et al. J. Sci. Indust. Res India* **12B,** 536 (1953)

40. Jones, D. L., and De La Juerga, J. *J. Lab. Clin. Med.* **45,** 87 (1955)

41. Kaplan, M. A., *et al. Antibiot. Ann.* (1959-60) p. 365.

42. Katsura, S., and Aoki, T. *Acta Med. Biol.* **7,** 1 (1959)

43. Kelly, R. G., and Buyske, D. A. *J. Pharmacol. Exptl. Therap.* **130,** 144 (1960)

44. Klinger, J., and Katz, R. *Gastroenterology* **41,** 29 (1961)

45. Knothe, H. *Arzn. Forsch.* **9,** 615 (1959)

46. Kunin, C. M., and Finland, M. *New. Engl. J. Med.* **259,** 999 (1958)

47. Kunin, C. M., *et al. ibid.* 147

48. Kunin, C. M., *et al. Antibiot. Ann.* (1959-60) p. 386

49. Leevy, C. M., *et al. ibid.* (1958-59) p. 258

50. Lepper, M. H. Aureomycin, New York, Med. Encycl, Inc., 1955

51. Lindenbaum, A., *et al. Arch. Biochem.* **52,** 110 (1954)

52. Loo, T. L., *et al. Science* **126,** 253 (1957)

53. Malek, P., *et al. Antibiot. Ann.* (1958-59) p. 221

54. Maynard, A. Ed. L., *et al. ibid.* (1953-54) p. 102

55. McCormick, J. R. D., *et al. J. Am. Chem. Soc.* **79,** 3461 (1957)

56. Mildberg, M. B., *et al. Antibiot. and chemother.* **4,** 1086 (1954)

57. Morris, W. E. *J. Am. Med. Assoc.* **172,** 1155 (1960)

58. Musselman, M. M. Terramycin. New York, Med. Encycl. Inc., 1956

59. *New. Engl. J. Med.* **258,** 97 (1958) ; **259,** 194 (1958)

60. O'Reilly, I., and Nelson, E. *J. Pharm. Sci.* **50,** 413 (1961)

61. P'an, S. Y., *et al. Ann. N. Y. Acad. Sci.* **53,** 238 (1950)

62. Petty, M. A. *Bact. Rev.* **25,** 111 (1961)

63. Pindel, M. H., *et al. J. Pharmacol. Exptl. Therap.* **122,** 61A (1958)

64. Posner, A. C., *et al. Antibiot. Ann.* (1954-55) p. 594

65. Privitera, F. *G. Mal. Infett. Parsit.* **11,** 88 (1959) ; cit. **B.A. 35,** 16675 (1960)

66. Purcell, E. M., *et al. Proc. Soc. Exptl. Biol. Med.* **85,** 61 (1954)

67. Roberts, M. S., *et al. Antibiot. Ann.* (1959-60) p. 424

68. Rogers, W. F., *et al. J. Lab. Clin. Med.* **45,** 87 (1955)

69. Ross, S., *et al. Antibiot. Ann.* (1959-60) p. 388

70. Saslaw, S. *New Engl. J. Med.* **264,** 1301 (1961)

71. Seto, J. F., and Lepper, M. H. *Antibiot. and Chemother.* **4,** 666 (1954)

72. Siedel, W., *et al. Munch. Med. Wochen.* **17,** 661 (1958) ; cit. *Am. J. Med. Sci.* **251,** 1 (1961)

73. Sirota, J. H., and Saltzman, A. *J. Pharm. Exptl. Therap.* **100,** 210 (1950)

74. Spector, W. S., ed. Handbook of toxicology, Philadelphia. Saunders, 1957. Vol. 2, pp. 51, 136, 181

75. Spitzy, K. H., and Hitzenberger, G. *Antibiot. Ann.* (1957-58) p. 996

76. Stephens, C. R., *et al. J. Am. Chem. Soc.* **76,** 3568 (1954)

77. Sugar, B., *et al. Am. J. Med.* **11,** 627 (1951)

78. Sweeny, W. M., *et al. New Engl. J. Med.* **263,** 620 (1960)

79. Ther, L., and Vogel, J. H. G. *Arnz. Forsch.* **9,** 63, (1959)

80. Trams, E. G., *et al. Antibiot. Med.* **1,** 677 (1955)

81. United States Dispensatory. 25th ed. Philadelphia, Pa., Lippincott, 1955

82. Van Meter, J. C., *et al. Proc. Soc. Exptl. Biol. Med.* **81,** 215 (1952)

83. Von Öttingen, W. F. *Am. J. Med.* **18,** 792 (1955)

84. Wagner, W. H., *et al. Antibiot. Ann.* (1959-60) p. 358

85. Welch, H. *Ann. N. Y. Acad. Sci.* **53,** 253 (1950)

86. Williams, R. T. Detoxication Mechanisms. 2d ed. rev. and enl. London, Chapman and Hall, 1959, p. 312

87. Wright, S., *et al. Antibiot. Ann.* (1953-54) p. 92

88. Zolotukhin, S. I. *Antibiotics* **4,** 324 (1959)

Studies on the Mode of Action of Antibiotics

I. HAMYCIN

S. RAMACHANDRAN

Research Laboratories, Hindustan Antibiotics Ltd., Pimpri, Near Poona

ACKNOWLEDGE of the mode of action of antibiotics is of fundamental as well as of practical interest. Firstly, an antibiotic whose mechanism of action is known could serve as a tool in the study of biochemical processes and secondly, the knowledge may help in tackling the problem of microbial resistance to antibiotics. In the last few years a number of papers have appeared on the mode of action of antibiotics. The antibiotics thus studied include penicillin, chloramphenicol, antimycin A, filipin, nystatin, ascosin, heptamycin, oleandomycin, tetracyclines, and a few others. Of these, the site of action of penicillin,[1] antimycin,[2] and ascosin[3] have been fairly well established. The studies on penicillin have helped our understanding of the cell-wall synthesis in gram positive bacteria ; antimycin is being used in the study of coupled electron transport mechanism[4]; chloramphenicol in the study of nucleic acid and protein synthesis[5] ; and filipin is contributing to the understanding of the permeability functions of the cell wall and cell-wall membranes.[6]

It is the purpose of this paper to present some of the results of the studies on the mode of action of hamycin. Hamycin, a heptaene, reported from this Laboratory, belongs to the large family of polyene antibiotics.[7] The antibiotic has high activity against certain fungi but exhibits little or no antibacterial properties.

MATERIALS AND METHODS

Saccharomyces cerevisiae which was used as the test organism, was grown in a Pfaudler 50 l. fermentor with aeration and agitation for 18 hr. at 28°. The composition of the medium used was : Glycerol, 10 ml. ; glucose, 10 g. ; yeast extract, 5 g. ; NaCl, 5 g. ; $MgSO_4.7H_2O$, 0.5 g. ; $FeSO_4$, 0.01 g. ; KH_2PO_4, 1.0 g. ; the volume was adjusted to 1.0 l. with distilled water, and the final pH was adjusted to 5.0. Paraffin oil, 2% by volume, was used as antifoam. The yeast cells were harvested with the use of a Sharples centrifuge and stored at 4°. These cells were used in the leakage studies.

In the growth studies, an 18-hr. old shake flask culture of *S. cerevisiae* was used for inoculating 50 ml. of the medium mentioned above in a 500-ml. conical flask. These flasks, after additions of appropriate amounts of hamycin* and

*The hamycin sample used in these experiments was non-crystalline.

cholesterol, were incubated at 28° on a rotary shaker (240 r. p.m., 2″ throw). At various time intervals the flasks were removed and growth was measured in a Lumetron at 650 mμ (red filter). The growth is expressed as percentage transmission of light at that wavelength.

In the leakage studies, 5 g. of the fresh yeast were washed thrice in distilled water and suspended in 100 ml. of 0.05*M* potassium citrate buffer, pH 6.5, in a 500-ml. conical flask and the appropriate amounts of hamycin and cholesterol were added. The flasks were incubated on the rotary shaker at 28°. At the fixed time intervals, samples were taken, centrifuged to sediment the cells and the supernatant liquid tested for amino acids and phosphates. Amino acids were determined by the standard ninhydrin method and the phosphates by the method of Fiske and Subbarow.[8]

Hamycin stock solution, 500 μg./ml. in 66% ethanol, was prepared fresh for each experiment. Cholesterol stock solution, 500 μg./ml. was prepared in absolute ethanol.

For studies on the interaction between hamycin and cholesterol in aqueous solutions, hamycin dissolved in 66% ethanol was added to 50 ml. of distilled water in a 500-ml. flask to give a concentration of 5 μg./ml. At that concentration of the antibiotic no turbidity of the solution was observed. In the experiments with sterol additions, cholesterol was added to give 5 μg./ml. The flask was then placed on the rotary shaker at 28° for 30 min. At the end of the period, samples were taken for study in a Beckman DU spectrophotometer, in the visible range.

RESULTS

Growth inhibition by hamycin

Hamycin in concentrations as low as 0.5 μg./ml. inhibits the growth of *S. cerevisiae* completely (Fig. 1) The incubation period was 24 hr. It is evident from fig. 1 that cholesterol vitiates the effect of hamycin. With increasing concentration of hamycin, the sterol-hamycin ratio needed to reverse the effect of the antibiotic increases. A 1:1 ratio of sterol is completely effective in its protective action only when the hamycin concentration is 0.5 μg./ml. When the antibiotic concentration is 1.0 μg./ml. the amount of sterol required to produce the same effect is 5 μg./ml.

Leakage from yeast cells

Tables 1 and 2 represent the results from experiment on leakage of amino-nitrogen and of phosphates from yeast cells under the influence of hamycin. Table 1 shows the effect of 1 μg./ml. hamycin on yeast cells with samples taken at different time intervals. *S. cerevisiae* grown under the experimental conditions apparently has a high content of endogenous amino-nitrogen that leaks out initially and is later absorbed even by the control cells (Table 1). The phosphate pool also seems to be high in the young yeast cells, and this also leaks out into the medium from the control. The effect of hamycin can be clearly seen over and above the normal leakage. The hamycin induced leakage is considerably less when cholesterol in a ratio of 1:1 is present. From table 1 it also appears that the cell leakage in the presence of hamycin does not commence till after 1 hr. Table 2 shows the effect of various concentrations of cholesterol on the cell leakage induced by three different concentrations of hamycin. The greater the concentration of hamycin the less effective the sterol protection.

Interaction of sterol with hamycin

Gottlieb *et al*[6, 9] and Lampen *et al*[10] have shown that in aqueous solutions, sterols in general appear to form complexes with polyene antibiotics. In our experi-

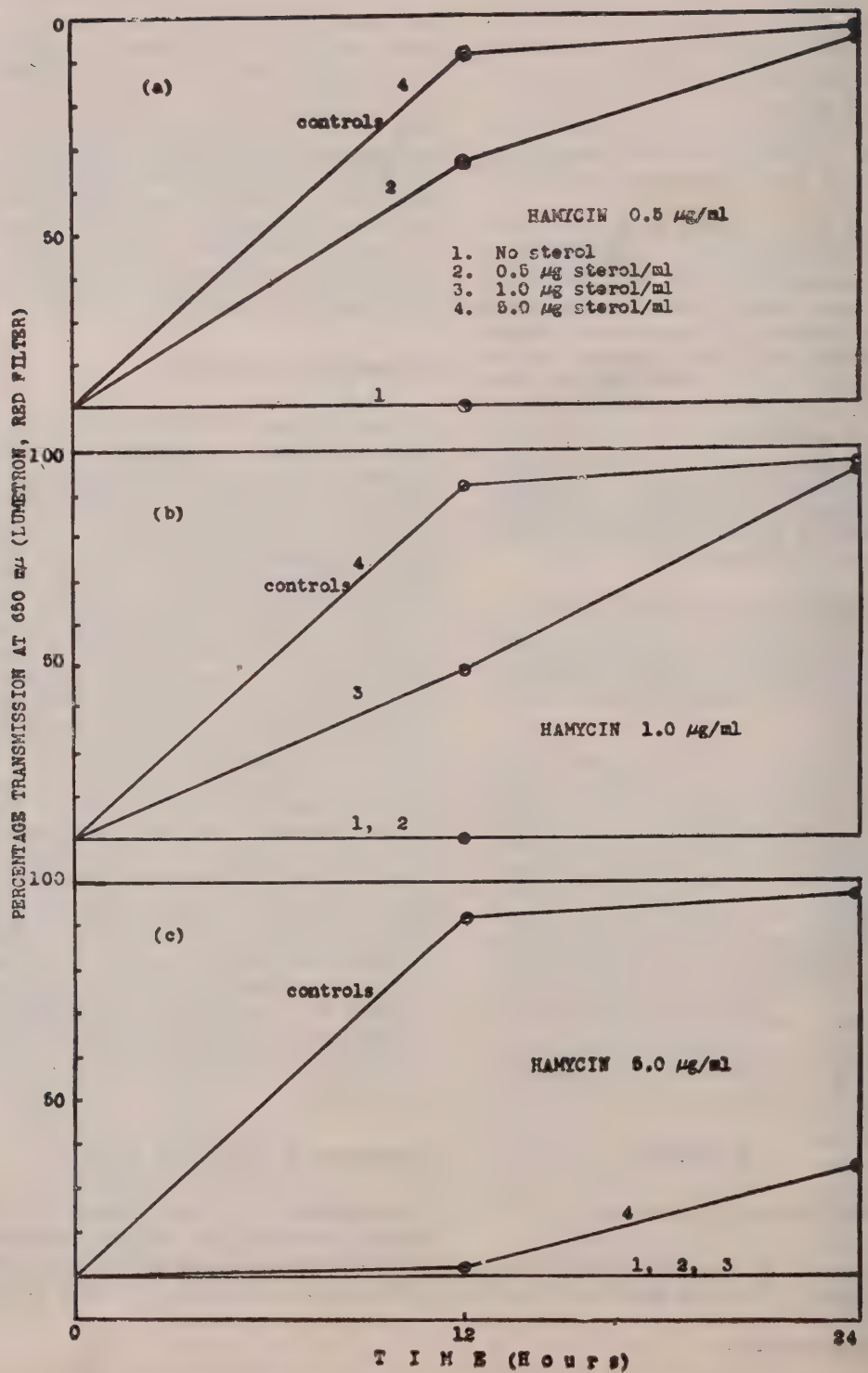


Fig. 1. Effect of hamycin with and without cholesterol on the growth of *Sacc. cerevisiae*

TABLE I

LEAKAGE OF AMINO-NITROGEN AND PHOSPHATE FROM *S. CEREVISIAE* INTO THE MILLIEU UNDER THE INFLUENCE OF HAMYCIN.

EACH FLASK CONTAINED 5.0 G. OF FRESH YEAST WASHED THREE TIMES IN DISTILLED WATER BY CENTRIFUGATION AND THEN SUSPENDED IN 50 ML OF 0.05M POTASSIUM CITRATE BUFFER, PH 6.5.

* μ moles of amino-nitrogen/ml. / TREATMENT	TIME (Hours) 0	1/2	1	7
CONTROL	2.1	1.14	0.96	0.88
+ Hamycin 1 μg/ml.	2.1	1.14	0.94	1.46
+ Hamycin 1 μg/ml. + Cholesterol 1 μg/ml.	2.1	1.14	0.94	1.16
μg PO_4/ml. / TREATMENT	0	1/2	1	7
CONTROL	75	160	166	177
+ Hamycin 1 μg/ml.	82	150	145	232
+ Hamycin 1 μg/ml. + Cholesterol 1 μg/ml.	80	150	145	164

* amino-nitrogen = *l*-glutamate equivalents.

ments, cholesterol exhibited similar effect when mixed with hamycin in an aqueous solution. This complexing effect is evidenced by lowering of the hamycin absorption maxima at 383 and 406 mμ by cholesterol (Fig. 2). This complex is apparently broken by the mere addition of ethanol in a 1 :1 ratio.

TABLE II

HAMYCIN-CAUSED LEAKAGE OF AMINO-NITROGEN AND PHOSPHATE FROM *S. CEREVISIAE* AND THE EFFECT OF CHOLESTEROL ON THIS LEAKAGE.

EXPERIMENTAL PROCEDURE SAME AS DESCRIBED UNDER TABLE I. SAMPLES WERE TAKEN AT THE END OF 6 HOURS INCUBATION AT 28° ON A ROTARY SHAKER.

Hamycin μg/ml.	Cholesterol μg/ml.	μ g PO_4/ml. of incubation medium	μ mole amino-nitrogen per ml. of incubation medium
0	0—'0' TIME	28	2.25
0	0	85	1.4
0	0 (2% Ethanol Control)	88	1.6
0	20	85	1.8
0.5	0	122	3.0
0.5	0.5	115	2.1
0.5	1.0	100	1.6
0.5	20.0	87	1.6
5.0	—	105	18.0
5.0	1.0	103	18.0
5.0	10.0	93	16.5
5.0	20.0	97	16.5
10.0	—	115	23.0
10.0	5.0	115	22.0
10.0	10.0	110	20.0
10.0	20.0	105	19.0

DISCUSSION

From the results presented in this paper it can be seen that hamycin is similar to filipin in its mode of action. The inhibition of the growth of *S. cerevisiae* by hamycin could be overcome by the addition of cholesterol in the ratio of 1 : 1 when the antibiotic concentration is low, and at a higher sterol ratio when the antibiotic concentration is high (5 μg./ml.) The protective effect exhibited by cholesterol could be considered under the following heads : (1) One of mere protection and no reversal; and (2) one of both protection and reversal. In aqueous solutions cholesterol interacts with hamycin and appears to form a complex (Fig. 2). The observed protective effect of sterol could be due to the *in vitro* inactivation of hamycin on complexing with sterol. However, the increased rate of cell division after a slow inhibited phase (curves b-3 and c-4, fig. 1) indicates that the sterol protection against hamycin might also include reversal of an existing antibiotic effect.

The cell-leakage effect produced by hamycin indicates that the antibiotic in some way affects the permeability of the yeast cell-wall membrane. With increasing concentrations of hamycin greater is the leakage. Cholesterol at proper concentrations is able to reduce or stop leakage. It is too early to interpret the leakage

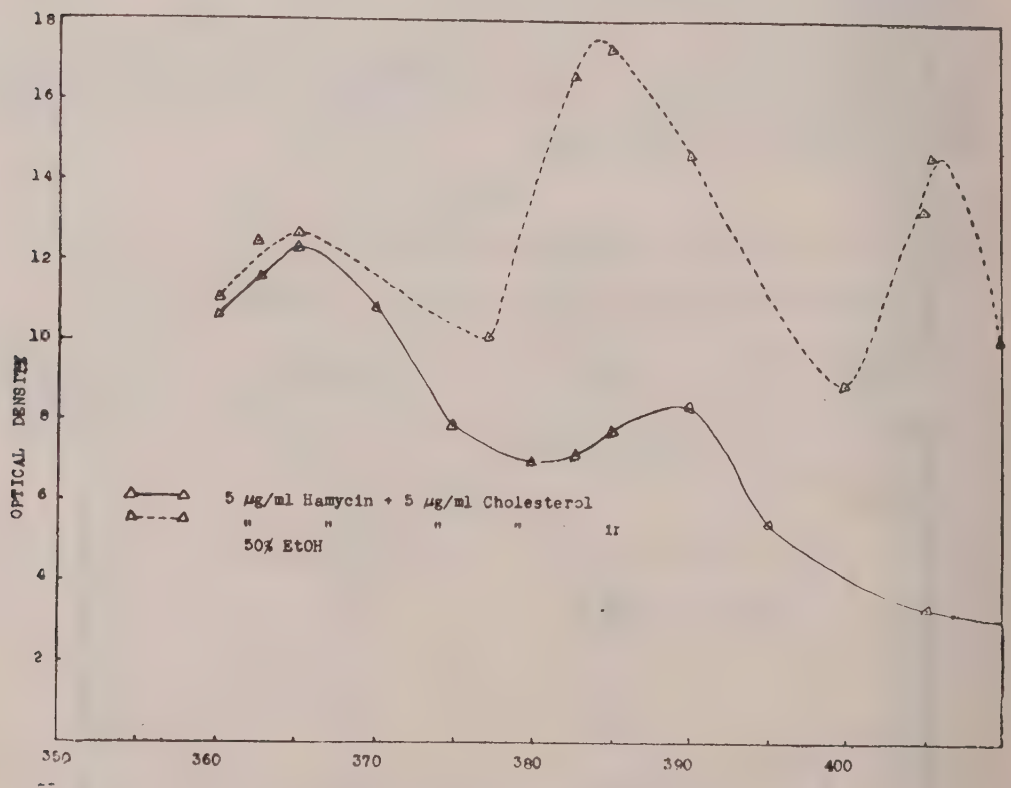


Fig. 2. Effect on cholesterol on the absorptivity of hamycin in the visible range of light

effect as either a direct or an indirect result of the antibiotic action.

SUMMARY

The results presented in this paper from studies on the mode of action of hamycin, a heptaene, can be summarized as follows.

1. Hamycin inhibits the growth of *Saccharomyces cerevisiae* in concentrations as low as 0.5 μg./ml. This inhibition is overcome by cholesterol. The ratio of cholesterol/hamycin needed to confer complete protection increases with increasing concentrations of hamycin.

2. In the presence of hamycin, *S. cerevisiae* cells leak out into the *milieu* compounds containing amino-nitrogen and phosphates. In the presence of cholesterol, however, hamycin does not cause this leakage.

3. In aqueous solutions cholesterol suppresses the absorption maxima of hamycin at 383 and 406 mμ. If this suppression indicates a complex formation between the sterol and hamycin, then the complex can be broken by the mere addition of ethanol at a ratio of 1 : 1 to the aqueous solution.

ACKNOWLEDGEMENTS

The author wishes to thank Dr. M. J. Thirumalachar for his interest in this investigation. The assistance rendered by Miss A. Gore in the growth experiments is also acknowledged.

REFERENCES

1. Park, J. T., and Strominger, L. *Science* **125,** 99 (1957)
2. Crane, F. L., and Glenn, J. L. *Biochim. Biophys. Acta* **24,** 107 (1957)
3. Ramachandran, S., *et al. Bact. Proc.* G. 65 (1961) ; Gottlieb, D., and Ramachandran, S. *Biochim. Biophys. Acta* (In press)
4. Gottlieb, D., and Ramachandran, S. *Biochim. Piophys. Acta* (In press)
5. Brock, R. D. *Bact. Rev.* **25,** 32 (1961)
6. Gottlieb, D., *et al. Phytopathology* **51,** 321 (1961)
7. Thirumalachar, M. J., *et al. Hindustan Antibiot. Bull.* **3,** 136 (1961) ; Bhate, D. S., *et al. ibid.* 139
8. Fiske, C. H., and Subbarow, Y. *J. Biol. Chem.* **66,** 375 (1925)
9. Gottlieb, D., *et al. Science* **128,** 361 (1958)
10. Lampen, J. O., *et al. J. Bact.* **80,** 200 (1960)

Octacillin in Acute Anterior Gonococcal Urethritis

B. B. GOKHALE, M. B. GHARPURE & H. E. POONAWALA

Department of Dermatology and Venereology, Sassoon Hospitals, Poona.

OCTACILLIN is the *N, N'*-di-*n*- octylethylenediamine salt of pencillin G. It is a colourless, crystalline compound, almost insoluble in water (0.033%). It is tasteless and almost odourless, but develops a pungent odour after long periods of storage.[1,2] In neutral aqueous suspension, octacillin is stable for a week, and at acid pH it is stable for a few hours at room temperature. In the solid form in properly closed containers it is stable for over two years.[3] *In vitro* tests indicate that all penicillin sensitive organisms are equally susceptible to octacillin. Earlier studies have shown that penicillin blood levels obtained after oral administration of octacillin are high and prolonged. In the present communication the clinical response of several cases of acute anterior gonococcal urethritis treated with octacillin are recorded. Penicillin blood levels obtained after various oral doses of octacillin were also observed and the results discussed in terms of the clinical response in the treated cases.

MATERIALS AND METHOD

Octacillin. *N, N'*-di-*n*-octylethylenediamine salt of penicillin G (Hindustan Antibiotics Ltd., Pimpri, Near Poona).

Test organism. *Neisseria gonorrhoeae* was chosen as the test organism. The studies were confined to gonococcal anterior urethritis incited by the organism. This coccus was chosen because of its marked susceptibility to penicillin, the common occurrence of the disease, easy diagnosis, and satisfactory identification and isolation of the causative organism. Further, clinical evaluation is simple and long follow-up is not necessary.

The patients. The patients studied were all males ranging in age from 18 to 35 years. Octacillin was tried in 16 patients with anterior gonococcal urethritis.

Dosage. Eleven patients were each given a single dose of 375 mg. of octacillin. Blood level studies indicated that with repeated divided doses of 375 mg. for 3 consecutive days, effective blood levels of penicillin were maintained to the end of 72 hr. Therefore, another group of 5 patients was treated in divided doses daily, 125 mg. T. D. S. at 8-hr. interval for 4 days. In this second group most of the clinical tests gave negative results after the first 48 hr. A further group of 5 cases was, therefore, treated with 375 mg. as an initial leading dose.

Criteria for assessment of results. Assessment of results of the clinical trials was based on the following criteria :

1. Clinical examination every 24 hr. ;

2. two glass test every 24 hr. ;

3. microscopic examination of smears initially after 24 hr. and then after 72 hr. ; and

4. cultures of the causative organism in a few cases, taken at random, done on the first day, fourth day, and a week later.

Assessment of cure. In this study a case was considered cured on

1. disappearance of visible urethral discharge and relief from disease symptoms ;

2. negative results with slide smears for a week ; and

3. two successive negative cultures at an interval of four days.

RESULTS

The blood levels obtained with various dosage schedules of octacillin are presented in Table I.

TABLE I

PENICILLIN BLOOD LEVELS AFTER ORAL ADMINISTRATION OF OCTACILLIN

S. No.	Age/Sex	Wt. in lbs.	Penicillin blood level in units/ml. after .. hr.			
			1	24	48	72
		(A) *125 mg. total, as a single dose*				
1	29/M	97	0.1	0.08	0.04	—
2	65/M	120	0.06	0.04	0.04	—
3	20/M	100	0.04	0.02	—	—
4	23/M	105	0.06	0.04	—	—
		(B) *250 mg. total, as a single dose*				
5	25/M	132	0.1	0.06	0.04	—
6	28/M	100	0.25	0.08	0.03	—
7	40/F	120	0.15	0.08	0.04	—
8	18/M	92	0.10	0.06	—	—
		(C) *275 mg. single dose*				
9	30/M	130	not taken	0.10	—	—
10	28/M	136	0.2	0.10	0.04	—
11	30/M	135	not taken	0.14	—	—
12	40/M	108	0.18	0.08	0.04	—
13	38/M	88	0.18	0.08	0.05	—
14	23/M	100	0.20	0.10	—	—
15	14/M	67	>0.20	0.16	—	—
16	14/M	66	>0.20	0.08	—	—
		(D) *375 mg. total dose for 4 days (125 mg. at 8-hr. interval)*				
17	23/M	100	0.06	0.15	0.30	0.06
18	18/M	100	0.08	0.01	0.02	0.04
19	21/M	122	0.08	0.01	0.06	0.03
20	30/M	120	0.04	0.08	0.10	0.04
21	20/M	123	0.06	0.01	0.20	0.04
22	55/M	112	0.08	0.12	>0.20	0.04

All the patients were examined daily for a period of 7 days. In the first group of 11 patients who received 375 mg. of octacillin as a single dose, the visible purulent discharge disappeared completely within 24 hr. in 9 cases, and in the remaining 2 cases the watery discharge persisted after 24 hr. The results of two glass test showed continuous improvement day by day. In all but one case no gonococci could be seen in smear made after 48 hr. In one case a few disintegrated cocci could be seen in one or two cells in one field. Even in this case a complete absence of the cocci was noted at the end of 72 hr. (Table II).

In the second group (Table III) all the 5 cases studied were initially positive both for smears and cultures. Each patient received 375 mg. of octacillin per day in equally divided doses for 4 days. The smears and cultures were negative after

TABLE II

GROUP 1, PATIENTS WITH ACUTE ANTERIOR GONOCOCCAL URETHRITIS, GIVEN 375 MG. OCTACILLIN STARTING ON FIRST DAY

S. No.	Age/Sex	Clinical findings					2 Glass test					Smears				
		B. P.	24 hr.	48 hr.	72 hr.	7 day	B.P.	24 hr.	48 hr.	72 hr.	7 day	B.P.	24 hr.	48 hr.	72 hr.	7 day
1	30/M	++++	++	+	—	—	+	—	—	—	—	+	+	+	—	—
2	40/M	+++	—	—	—	—	+	—	—	—	—	+	—	—	—	—
3	21/M	+++	—	—	—	—	+	—	—	—	?	+	—	—	—	—
4	26/M	++++	—	—	—	—	+	—	—	—	—	+	—	—	—	—
5	26/M	++	—	—	—	—	?	—	—	—	?	+	—	—	—	—
6	20/M	++++	++	—	—	—	+	?	—	—	—	+	+	—	—	—
7	33/M	++++	++	—	—	—	+	+	—	—	—	+	—	—	—	—
8	28/M	++++	—	—	—	—	+	—	—	—	—	+	—	—	—	—
9	30/M	+++	—	—	—	—	+	—	—	—	—	+	—	—	—	—

TABLE III

GROUP 2, PATIENTS WITH ACUTE ANTERIOR GONOCOCCAL URETHRITS, GIVEN 125 MG. OCTACILLIN T. D. S. FOR 4 DAYS

S. No.	Age/Sex	Clinically : Discharge					Smears					Cultures				
		B. P.	24 hr.	48 hr.	72 hr.	96 hr.	B. P.	24 hr.	48 hr.	72 hr.	96 hr.	B. P.	24 hr.	48 hr.	72 hr.	96 hr.
1	24/M	++++	++	—	—	—	+	+	—	—	—	+	+	—	—	—
2	34/M	++++	—	—	—	—	+	—	—	—	—	+	—	—	—	—
3	18/M	++++	+++	—	—	—	+	—	—	—	—	+	+	—	—	—
4	22/M	++++	++	—	—	—	+	+	—	—	—	+	—	—	—	—
5	24/M	++++	—	—	—	—	+	—	—	—	—	+	—	—	—	—

TABLE IV. GROUP 3, PATIENTS WITH ACUTE ANTERIOR GONOCOCCAL URETHRITIS GIVEN 375 MG. OCTACILLIN AS INITIAL LEADING DOSE

S. No.	Age/Sex	Clinically : Discharge					2 Glass test					Smears					Cultures				
		B. T.	24 hr.	48 hr.	72 hr.	5th day	B.T.	24 hr.	48 hr.	72 hr.	5th day	B.T.	24 hr.	48 hr.	72 hr.	5th day	B.T.	24 hr.	48 hr.	72 hr.	5th day
1	17/M	+++	+	+	+	±	+	±/+	+	+	−	+	+	−	−	−	+	+	+	−	−
2	28/M	++	+	−	:	:	+	±/+	±/+	:	:	+	±/+	−	:	:	+	−	−	:	:
3	30/M	++	+	+	+	±/+	+	+	+	+	−	+	+	−	−	−	+	−	−	−	−
4	42/M	++	+	−	−	−	+	+	±	−	−	+	+	+	+	−	+	+	−	−	−
5	33/M	++	−	−	−	−	±/+	−	−	−	−	+	+	−	−	−	+	−	−	−	−

24 hr. in one case, while in 3 cases smears and cultures were negative in the 72 d and 96th-hr. samples respectively. The first case of this group failed to turn up for the follow-up.

Compared to the second group, the results obtained with the third group of 5 cases (Table IV), each receiving 375 mg. as an initial leading dose, were somewhat irregular. (Care was taken to see that the drug was taken in the out-doors department itself thereby minimizing the chances of the patient misusing the preparation). Nevertheless, all the patients showed conclusively negative results in all the tests by the fifth day.

In the last two groups, two patients had doubtful discharge, which was negative for smears. It is interesting to note that the smears were negative in two patients at the end of 48 and 72 hr. respectively.

DISCUSSION

Research on various antibiotic preparations has as one of its motivating factors that a higher and longer blood level obtained with a preparation would also enhance its therapeutic potential. Clinical confirmation of this hypothesis would be reflected in the more rapid inhibition of the invading organism, lower dosage and shorter course of medication. Penicillin preparations show considerable differences in their *in vitro* antibacterial activity, and these differences raise the question whether sensitivity tests performed as a guide to treatment can be restricted to any one assumed standard penicillin such as penicillin G. Garrod[4,5] has pointed out that antibacterial activity demonstrable *in vitro* is only one of the properties determining the therapeutic value of an antibiotic, and the significance of this property should not be exaggerated. It is known, for example, that there are several antibiotics as active as chloramphenicol against the typhoid bacilli *in vitro* but are less effective in the

body. In the case of oral penicillins also disparities may exist between the *in vitro* experiments and the environmental conditions obtained in the body which would influence the action of the preparations. These factors seem to point to the fact that clinical correction of a disease by a drug may not completely correlate with the blood levels obtained with the compound. Various investigators have, in fact, pointed out that although comparably longer and even higher blood levels are obtained with some orally administered pencillins, such as penicillin V than with injected penicillin, the clinical response in patients receiving oral penicillin has been far from satisfactory. Some investigators have, therefore, shown a definite preference for injectable penicillin.

From the clinical trials of octacillin described above we feel that the clinical response observed is comparable with that seen in cases treated with injectable penicillin. Further studies on octacillin treatment of other diseases incited by organisms sensitive to penicillin would be most useful.

SUMMARY

A series of cases of acute anterior gonococcal urethritis were treated with octacillin, an orally administered penicillin. The clinical cure and negativity in smears and cultures were observed with various dosage schedules of the drug. The results obtained were satisfactory.

ACKNOWLEDGEMENTS

Thanks are due to Dr. N. S. Deodhare, Pathology Department, B. J. Medical College, and Mr. A. A. Padhye, Department of Dermatology and Venereology, Sassoon Hospitals, Poona, for their cooperation in making the smears and estimation of blood levels, respectively. The assistance rendered by the Professor of Pathology, B. J. Medical College, is also acknowledged.

Octacillin was obtained through the courtesy of the Managing Director, Hindustan Antibiotics, Ltd., Pimpri, Near Poona.

REFERENCES

1. Vyas, G. N., and Dhopate, S. G. *Curr. Sci.* **25,** 356 (1956)
2. Vyas, G. N., and Dhopate, S. G. *J. Sci. Industr. Res.* (*India*) **18C,** 6 (1959)
3. Vyas, G. N., and Dhopate, S. G. *Antibiot. and Chemother.* **9,** 203 (1959)
4. Garrod, L. P. *Brit. Med. J.* **1,** 527 (1960)
5. Garrod, L. P. *ibid.* **2,** 1695 (1960)

Colorimetric Method for Determination of Penicillin

II. DETERMINATION OF PENICILLIN IN PROCESS SAMPLES

G. R. DESHPANDE & S. S. KARMARKAR

Research Laboratories, Hindustan Antibiotics Ltd., Pimpri, Near Poona

In Part I[1] we described the colorimetric estimation of penicillin in procaine penicillin G with phosphomolybdic acid as the reagent. Further applications of the method to process samples in the manufacture of penicillin are presented in this communication.

MATERIALS AND METHOD

The equipment and reagents used were the same as described in our earlier paper.[1]

The calibration curve (Fig. 1) was plotted following the procedure described previously.[1]

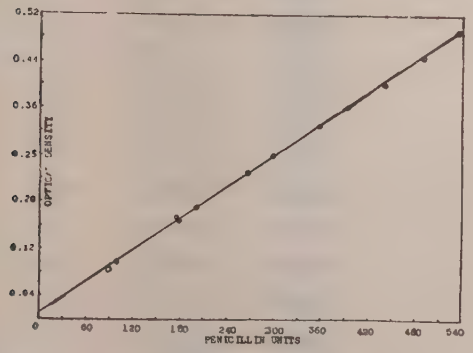


Fig. 1. Concentration curve (Wavelenght 270 mμ).

Estimation of penicillin in process samples

Fermentation broth.— The attempt to estimate penicillin in the fermentation broth directly was not completely successful because the raw materials used in the fermentation media give colour with phosphomolybdic acid. However, penicillin could be estimated successfully in broth samples when synthetic media was employed.

Fermentation broth, synthetic media.— Filtered broth (0.1 ml.) was used directly for development of the colour. If the colour is too deep the solution is diluted to double or triple its volume. The data obtained is presented in Table I.

TABLE I

PENICILLIN IN FERMENTATION BROTH, SYNTHETIC MEDIUM

No.	Hours	Penicillin in units/ml.		% variation
		Iodometry	Colorimetry	
1	16	nil	nil	nil
2	24	nil	nil	nil
3	41	50	50	0
4	61	110	120	+9.0
5	73	210	200	—5.0
6	87	200	200	0
7	96	200	220	+10.0
8	111	360	350	—3.0
9	135	680	700	+2.6
10	144	620	600	—3.0
11	170	1,020	1,000	—2.0

Acetate extract.— An accurate estimation of penicillin in the acetate extract directly with the colorimetric reagent in the organic phase (10 per cent solution of phosphomolybdic acid in a mixture of 50 per cent *iso*butanol and 50 per cent benzene) did not succeed as the solvent itself gave colour with the reagent. However, rough but reliable estimations could be made as follows.

Acetate extract (2 ml.) was shaken with 0.1 *M* phosphate buffer, pH 8 (10 ml.). After centrifugation the acetate layer was discarded, and the buffer extract (0.1 ml.) was used for the estimations, with buffer (0.1 ml.) as the blank. The data obtained are given in Table II.

TABLE II

PENICILLIN IN ACETATE EXTRACT

No.	Penicillin in units/ml.		% variation
	Iodometry	New colorimetry	
1	11,550	12,000	+3.89
2	6,440	6,250	—2.9
3	13,700	14,000	+2.1
4	13,065	13,010	—0.42
5	11,890	12,000	+1.1
6	34,470	35,100	+0.14
7	11,730	11,500	—1.9
8	11,560	11,350	—1.9
9	9,260	9,310	+0.54
10	8,920	9,050	+1.45
11	11,700	11,830	+1.1
12	8,200	8,160	—0.48
13	9,760	9,750	—0.09
14	25,870	25,900	+0.21
15	13,860	14,100	+0.17
16	12,700	12,620	—0.63
17	11,210	11,000	—1.89
18	13,170	13,200	+0.26
19	78,420	77,400	—1.3
20	78,790	79,800	+1.2

In the case of concentrated acetate extracts, the solution (2 ml.) was extracted with buffer, pH 8 (0.1 *M*, 98 ml.), and the extract (0.1 ml.) was used for the estimations, with buffer (0.1 ml.) as the blank.

Potassium extracts.— The extract (1 ml.) was diluted to 100 ml. with distilled water in a volumetric flask, and the diluted solution (10.1 ml.) was used for the determinations. The results of the estimations are given in Table III.

TABLE III

PENICILLIN IN POTASSIUM EXTRACTS

No.	Penicillin in units/ml.		% variation
	Iodometry	New colorimetry	
1	2,90,000	3,00,100	+3.0
2	2,64,000	2,80,000	+0.6
3	3,43,200	3,31,000	—3.0
4	3,20,000	3,18,000	—0.76
5	3,26,700	3,20,000	—0.2
6	3,48,400	3,38,000	—2.8
7	3,68,500	3,63,000	—1.3
8	1,80,900	1,85,500	+2.4
9	3,31,500	3,40,200	+2.5
10	2,68,800	2,67,200	—0.59
11	3,57,500	3,58,200	+0.02
12	3,31,500	3,40,200	+2.5
13	3,38,000	3,36,500	—0.44
14	3,38,000	3,40,000	+0.58
15	3,31,500	3,32,600	+0.33
16	2,99,000	3,00,100	+0.33
17	3,36,600	3,35,300	—0.39
18	3,56,400	3,55,800	—0.02
19	3,32,000	3,34,050	+0.59
20	3,03,000	3,05,200	+0.72

Potassium acetate mother liquors.— Estimations of penicillin in these mother liquors could not be done because the presence of large quantities of salts interfere with the colorimetric measurements. This was established by adding a drop of saturated solution of potassium acetate to the pure penicillin solution and noting the reduction in the colour intensity as compared with the blank.

Sodium extracts.— The extract (0.5 ml.) was diluted to 100 ml. with distilled water and 0.1 ml. of this solution was used in the estimations (Table IV).

TABLE IV

PENICILLIN IN SODIUM EXTRACTS

No.	Penicillin in units/ml.		% variation
	Iodometry	New colorimetry	
1	5,67,600	5,37,200	—5.3
2	4,02,600	4,02,000	—1.4
3	6,40,200	6,35,000	—8.1
4	5,21,400	5,23,000	+0.31
5	4,22,400	4,12,000	—2.4
6	6,02,000	5,95,000	—1.1
7	4,80,000	4,91,050	+2.2
8	3,50,000	3,60,000	+2.8
9	7,03,500	7,08,000	+6.3
10	5,52,750	5,48,600	—7.4
11	3,57,500	3,61,500	+11.1
12	7,37,000	7,32,500	—6.0
13	5,29,300	5,30,200	+0.16
14	4,75,700	4,70,500	—1.1
15	7,23,600	7,25,200	+2.2
16	5,82,900	5,84,000	+0.18
17	5,15,900	5,14,200	—0.32
18	7,32,600	7,33,000	+0.05
19	5,47,800	5,45,200	—0.47
20	5,04,900	5,00,300	—0.84

Spent acetate.— Ten ml. of the solvent was extracted with 0.1 *M* phosphate buffer, pH 8 (10 ml.) ; 0.1 ml. of the buffer was used for the determinations. The results are presented in Table V.

TABLE V

PENICILLIN IN SPENT ACETATES

No.	Penicillin in units/ml.		% variation
	Iodometry	New colorimetry	
1	3,300	3,200	—3.0
2	180	180	0
3	160	180	+2.8
4	200	210	+4.7
5	1,878	1,800	—4.1
6	560	600	+6.6
7	540	600	+10.0
8	100	120	+20.0
9	260	250	—4.0
10	130	120	—7.6
11	200	200	0
12	260	250	—4.0
13	470	480	+2.1
14	200	180	—10.0
15	130	150	+1.3
16	130	150	+1.3
17	330	400	+17.0
18	200	200	0
19	250	260	+4.0
20	150	150	0

Butanol mother liquors.—Butanol mother liquor (0.1 ml.) was used directly for the estimation of penicillin with fresh butanol

(0.1 ml.) as blank. The data is given in Table VI.

TABLE VI

PENICILLIN IN BUTANOL MOTHER LIQUORS

No.	Penicillin in units/ml.		% variation
	Iodometry	New colorimetry	
1	17,820	17,300	—2.8
2	18,400	18,700	+1.6
3	15,150	15,600	+2.8
4	13,000	13,300	+2.2
5	1,320	1,300	—1.5
6	18,820	17,020	—5.6
7	17,800	16,800	—5.5
8	21,120	25,000	+1.5
9	1,980	1,920	—3.3
10	17,000	16,500	—2.9
11	14,740	15,000	—2.9
12	30,490	29,820	—2.2
13	13,280	13,650	+2.9
14	26,130	25,020	—4.2
15	22,400	21,950	—2.0
16	32,160	32,100	—0.18
17	20,100	21,200	+5.1
18	26,130	25,850	—0.96
19	18,200	18,100	—0.54
20	17,560	18,000	+2.4

Crystal washes.— Dilution of the sample is made in such a way that 0.1 ml. of the solution contains less than 500 units of penicillin. Results of penicillin estimations in the washes are presented in Table VII.

TABLE VII

PENICILLIN IN CRYSTAL WASHES

No.	Penicillin in units/ml.		% variation
	Iodometry	New colorimetry	
1	11,220	10,700	—4.6
2	9,900	9,800	—0.97
3	21,450	20,800	—0.3
4	13,200	13,000	—1.5
5	13,800	14,000	+1.4

General procedure for the estimation of penicillin in process samples.

To the test solution (0.1 ml.) and the blank solution (0.1 ml.) sufficient water is added to make the volume equal to 1 ml. in each case. Phosphomolybdic acid reagent (0.4 ml.) is added to each test tube, and the samples are heated on a boiling water bath for exactly 10 min. The test tubes are cooled to room temperature and the solution diluted to the 10-ml. mark with distilled water. The absorbance is read off at 720 mμ using 1 cm. cell in the Beckman spectrophotometer or 1.5 cm. tube in the Coleman Universal spectrophotometer. The unitage of penicillin is calculated from the corresponding calibration curve.

In each case a number of samples have been analysed and the results compared with iodometric assay.

DISCUSSION

The time factor is of considerable importance in routine assays in industrial practice. From this point of view the present assay method has a definite advantage over other methods, especially iodometry which is commonly used in the estimation of penicillin. With the present

method a number of samples can be handled at a time and the assay is complete within 20 min. The method is also sensitive and can estimate penicillin from 10 to 500 units in the final solution. Iodometry usually needs more time and higher unitage. The accuracy of the new colorimetric method is evident from the comparative data presented in the Tables.

SUMMARY

Penicillin has been estimated colorimetrically, with phosphomolybdic acid as the reagent, in samples from various stages in the production of penicillin carried out on a laboratory scale. The method has advantages in routine estimations of penicillin as it is fast and the results obtained compare well with those obtained by the iodometric method. The method did not succeed in penicillin estimations in broth samples where complex media were used, and also in the case of potassium acetate mother liquors.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. M. J. Thirumalachar and Dr. D. S. Bhate for their interest in the work.

REFERENCES

1. Deshpande, G. R., and Karmarkar, S. S. *Hindustan Antibiot. Bull.* **3**, 174 (1961)

Physico-Chemical Data on Antibiotics

II. ANTIBIOTICS PRODUCED BY *ACTINOMYCETES*

3. Antibiotics with antiviral activity

In two earlier tabulations[1, 2] of the physico-chemical data on antibiotics, antiviral compounds produced by fungi and bacteria have been included. In a review on the infectious nature of ribonucleic acid of certain viruses Rawal[3] has listed the antibiotics active against viruses and phages. In the present compilation the physico-chemical and antiviral properties of compounds produced by actinomycetes are tabulated (Table II).

The serial numbering is continued from the previous paper in this series,[4] and compounds from actinomycetes listed earlier are cross-referred to with additional information, if any. The antibiotics are grouped under the following heads :

Amino acid and polypeptide-type

Nitrogen heterocyclic compounds

Quinonoidal compounds

Pigments

Dilactones

Cycloheximide-type

Alicyclic carboxylic acids

Streptothricin-related compounds

Tetracyclines

Other compounds

An asterisk against the name of an antibiotic indicates that its partial or full structure is known.

Table I is an index to the antibiotics by the virus inhibited. Melting point, ultraviolet absorption maxima, and empirical formulae indices are provided as in previous compilations of this series.

Library A. NEELAMEGHAN
Hindustan Antibiotics Ltd.
Pimpri, Near Poona.

REFERENCES

1. Neelameghan, A. *Hindustan Antibiot. Bull.* **2**, 13 (1959)
2. Neelameghan, A. *ibid.* 54
3. Rawal, B. D. *ibid.* **3**, 55 (1960)
4. Neelameghan, A. *ibid.* 152

TABLE I

VIRUSES INHIBITED BY THE ANTIBIOTICS

Virus inhibited	Antibiotic S. No. (Table II)
Cowpox	179, 240
Encephalitis	
Eastern	75, 234
Japanese B	210, 232
Tick	213
Western	75
Equine infectious anaemia	83 (?)
Herpes simplex	181, 240
Influenza	79, 89, 113, 114, 212, 213, 216, 217, 222, 236, 243
Influenza A	211, 214, 215, 218, 244
Influenza B	211, 241
Influenza FM-1	242
Influenza PR-8	106, 110, 234, 244
Lymphogranuloma venereum	209, 225-231
Meningopneumonitis	219, 225-231
Mumps	211
New Castle disease	211, 216, 217
Phages	62, 179, 213, 224, 233-235, 238-240, 242, 243
Pneumonitis	225-231
Poliomyelitis	75, 234, 240
Rabies	237, 240
Silkworm jaundice	213, 220, 221, 223
Smallpox	212, 213, 221, 244
Tobacco mosaic virus	86, 212, 244, 245

TABLE II. ANTIVIRAL ANTIBIOTICS PRODUCED BY ACTINOMYCETES

S. No.	Antibiotics, and Producing organism	Chem. nature. Mol. composition	M. p.	α in mμ λmax	$[\alpha]_D$	Colour reactions, functional group tests.	Antibiotic activity. References
1	2	3	4	5	6	7	8
		AMINOACID & POLYPEPTIDE TYPE					
209	*CHLORAMPHENICOL *S. venezuela* By synthesis	Colourless elongated plates or fine needles. Neutral. $C_{11}H_{12}N_2O_5Cl_2$	149.7—150.7 (corr.)	278	—25.5 (ethyl acetate). +19(EtOH)	Neg. $FeCl_3$, Sakaguchi, Pauly, Molisch, biuret, Benedict. Non-ionic chlorine.	Active against gram+ gram—bacteria, rickettsiae, large viruses such as viruses of lymphogranuloma venereum and psittacosis in mice or embryonated eggs, and experimental airborne mouse pneumonitis virus infection in mice. *H. T.* **2**
210	CEPHALOMYCIN *S. tanashiensis* var. *cephalomyceticus*	Brownish amorphous powder. Acidic amphoteric. C, 55.39; H, 6.66; N, 9.93 (9.3 by kjeldahl)		None (H_2O). Sh. 255-260, 640-650, 740-775 (Folin's reagent)		Salted out from aq. soln. at ¾ saturation with $(NH_4)_2SO_4$. Pptd. by alum, and picric, flavianic, phosphotungsticacids, $CuSO_4$, $ZnCl_2$, $FeCl_3$, trichloroacetic acid. Pos. Sakaguchi, biuret, ninhydrin, diazo, Folin. Neg. Fehling, Molisch, xanthoproteic. Acid hydrolysates contain leucine, phenylalanine, valine, tyrosine, alanine, threonine, glutamic acid, glycine, arginine, aspartic acid, 3 other chromatog. spots.	Culture filtrates active against gram + bacteria. Lyophilized powder active only against *D. pneumoniae*, *Sh. dysenteriae*, *Sacc. sake*, Japanese B encephalitis virus inhibited *in vitro* and *in vivo*. *J. Antibiot.* (*Jap.*) **13A**, 143 (1960)
		NITROGEN HETEROCYCLIC COMPOUNDS					
	*NETROPSIN (*See* 179)						

1	2	3	4	5	6	7	8
211	*NOFORMICIN *Nocardia formica*	[2-Imino-5-(2'-amidinoethyl-carbamoyl) pyrrolidine. $C_{17}H_{34}N_{10}O_5(SO_4)_2$ for cryst. sulphate.	HCl, 265 dec. Sulph., 263 dec.	None characteristic.		Dialyzable. Ammonia and glutamic acid among hydrolysis products.	Effective against viruses of influenza A, B, swine influenza in mice, mumps virus and Newcastle disease virus in embryonated eggs. Suppresses TMV in various plants. *H. T.* **2.** Indian patent 52431.
	*PUROMYCIN (*See* 181)						Conc. of 2 mg./ml. partially inactivates virus of herpes simplex.
				QUINONOIDAL COMPOUNDS			
	*CINERUBIN A (*See* 113)						
	*CINERUBIN B (*See* 114)						
				PIGMENTS			
212	ANTIVIRUBIN *S. longisporuber* (mycelium)	Bright red pigment with properties of a dye				Little inactivation by blood serum.	Inactivates TMV, influenza and smallpox viruses. Staphylococci, *B. mycoides* and hay bacilli inhibited. *Antibiotiki* **1,** No. 4, 62 (1956)
213	VIOLARIN *S. violaceus*	Amorphous bright red pigment. Acid base indicator. $C_{22\text{-}24}H_{32\text{-}34}O_{8\text{-}9}$; mol.wt. 443, 446, 419.		498, 530 (n-BuOH) 499, 500 (dimethylformamide)		Aq. soln. with NaOH gives blue colour. Nascent H_2 decolourizes acid tinctures, colour restored on exposure to air. No ppt. with PbAc. Not decolourized by H_2O_2.	Active against gram+ bacteria, and virus of *Bombyx mori* yellow; influenza and smallpox (in chick embryos), tick encephalitis virus (exptl. animals), and phages. *Antibiotics* (*Trans.*) **4,** 517 (1959). *Antibiotiki* **3,** No. 3,18(1958)-

1	2	3	4	5	6	7	8
214	ANTIBIOTIC 719 *S. violaceous* 719	Dark red powder. Pigment, insol. in H_2O, sol. in EtOH.					Partly effective against influenza A virus *in vitro*, and highly effective in chick embryos. *Antibiotics* (*Trans*) **4**, 282 (1959)
215	ANTIBIOTIC M-II *S. violaceous 1*	Dark red powder. Pigment, insol. in H_2O, sol. in EtOH					Activity similar to that of Antibiotic 719 (214) *Antibiotics* (*Trans*) **4**, 282 (1959)
			DILACTONES				
	*ANTIMYCIN (*See* 106) (ANTIMYCIN A)						Low conc. (0.013 μg./ml.) inhibit propagation of PR-8 influenza virus, but do not destroy preformed virus.
			CYCLOHEXIMIDE-TYPE COMPOUNDS				
216	NIROMYCIN A *S. albus*	White amorphous powder. Hygroscopic. Neutral. $C_{14}H_{21}NO_4$	98-105		Inact. in 1% EtOH soln.	Pos. Tollens, 2, 4-DNPH. Neg. ninhydrin, $FeCl_3$, Fehling, Benedict, Molisch, biuret, $KMnO_4$ Some similarities to fermicidin (83)	Inhibits New Castle virus in tissue culture of chick embryo cells, and influenza virus in embryonated eggs. *J. Antibiot.* (*Jap.*) **13A**, 97, 110 (1960)
217	NIROMYCIN B *S. albus*	White hygroscopic cryst. C 62.57, H 7.54, N 5.31.	47-67. Semicarbazone 175-76.		Inact. in 1% EtOH soln.	Similar to niromycin A. Resembles cycloheximide, fermicidin, streptovitacins (86-88)	Activity similar to that of niromycin A. *J. Antibiot.* (*Jap.*) **13A**, 97, 110 (1960)
	FERMICIDIN (*See* 83)						
	CYCLOHEXIMIDE (*See* 86)						Effective against TMV *Phytopath.* **49**, 502 (1959)

1	2	3	4	5	6	7	8
		ALICYCLIC CARBOXYLIC ACIDS					
218	*MYXOVIROMYCIN (AMIDINOMYCIN) *Streptomyces* sp. related to *S. flavochromogenes*	Sulphate : Colourless fine needles. N-(2′-amidinoethyl)-3-aminocyclopentanecarboxamide. $C_9H_{18}N_4O$	Sulphate : 284-290 in sealed tube; 289-92 under microscope	205 (O.1*N* HCl) 219 (0.1*N* NaOH) 207 211-212	-3.6 (25°) -3.9 (21°)	Rf (BuOH-AcOH-H_2O), 0.25-0.27	Inhibits spore forming bacteria, influenza A virus *Jap. J. Microbiol.* **1,** 85 (1957), *J. Antibiot. (Jap.)* **13A,** 362 (1960) *ibid.* **14A,** 103, 163, 165, 251 (1961)
		STREPTOTHRICIN-RELATED COMPOUNDS					
219	*HYGROMYCIN (HOMOMYCIN) *S. hyroscopicus; S. noboritoensis*	White powder. Weakly acidic, pka 8.9. Composed of 3, 4-dihydroxy-∝-methylcinnamic acid, sugar, and neo-inosamine. $C_{23}H_{29}NO_{12}$	105-09, dec. gradually above 160	272, 214 (dil. HCl); 254, 286, 323 (dil. alkali)	-126 (c, 1 H_2O, 25°)	Pos. Folin-Ciocalteu, Benedict, Fehling, diazo, Nessler, Tollens, indole, carbazole. Neg. $FeCl_3$, anthrone, Molisch, maltol, ninhydrin, biuret. Strong I.R. absorption at 3.0, 5.84, 6.08, 6.22, 6.62 μ.	Activity in mice infected with meningopneumonitis virus. Inhibits gram+, gram—bacteria, some fungi and actinomycetes. *H. T.* **2.** *J. Antibiot. (Jap.)* **9A,** 225 (1956); *ibid.* **10A,** 21, 36, 160. (1957), *J. Am. Chem. Soc.* **79,** 120 (1957) ; *Antibiot. Ann.* (1956-57) 883.
220	GRASSERIOMYCIN *S. griseolavendulae*	HCl : Faint yellow.	Helianthate : 215-25 dec. Reineckate : 187-90 dec.	HCl has no specific absorption.		Resembles streptothricin in most reactions. Aq. HCl soln. gives pos. Molisch, Fehling, Tollens, ninhydrin ; neg. biuret, Sakaguchi, xanthoproteic, Millon, Adamkiewicz, Liebermann, Neubauer, $FeCl_3$. I. R. 3.05, 3.4, 4.3, 5.88, 6.07, 6.20, 6.45,7.02, 7.38, 7.6, 8.48, 8.7, 8.79, 9.34, 9.8, 10.52 μ.	Inhibits virus of silk worm jaundice, gram +, gram—bacteria and some fungi. *H. T.* 2. *CA* **52,** 20918d (1958)

1	2	3	4	5	6	7	8
221	VIRUSIN 1609 *Streptomyces* sp.	Crude ppn. amorphous, hygroscopic, cream coloured. HCl : C, 37.94, 37.34 ; H, 7.1, 7.08 ; N, 15.52, 15.53 ; Cl, 18.23, 18.01				Sol. in H_2O, org. solv. Resembles luridin (223), streptothricin-like. Forms picrate, helianthate. Neg. matol; pos. Sakaguchi Pauly, ninhydrin (neg. for pure product HCl), biuret, Molisch.	Effective against virus of silk worm jaundice, and smallpox in chick embryos. Inhibits gram+ gram—bacteria. *Antibiotics* (*Trans.*) **4**, 138 (1959) ; *Antibiotiki* **2**, No. 3, 14 (1957).
222	POLYMYCIN Actinomycete			281		Resembles streptothricin and carzinophillin (170-72)	Active against gram+, gram—acidfast bacteria, tumours, influenza virus. *CA*.**55**, 10577d (1961).
223	LURIDIN *S. lurides* (Grisemin producer)	HCl: C, 37.87, 37.55; H, 7.16, 7.31; N, 15.67, 15.98 ; Cl 18·8, 19·02				Crude luridin has properties and colour reactions similar to those of virusin (221). Streptothricin-type.	Inhibits virus of silk worm jaundice, gram+, gram-bacteria. *Antibiotics* (*Trans.*), **4** 138 (1959).
224	PHAGOMYCIN *Streptomyces* sp. resembling *S. griseolus*	Reineckate: C, 12.38; H, 6.79; N, 12.14; S, 14.34; Cr, 5.24.	Reineckate : 166-70	HCl : End absorption as for Streptomycin group		Pos. ninhydrin. Neg. biuret, xanthoproteic, Sakaguchi, maltol, Molisch, Millon, Fehling, Hopkins-Cole, Tollens, $FeCl_3$.	Inhibits bacterial viruses and phages. *J. Antibiot.* (*Jap*) **9A**, 108 (1956).
		TETRACYCLINES					
225	*6-DEMETHYL-TETRACYCLINE *S. aureofaciens* (mutant)	$C_{21}H_{22}N_2O_8$	HCl hemihydrate : 203-09 dec.		HCl hemihydrate : -259 (0.5% in 0.1 *N* H_2SO_4)		Antibacterial activity similar to that of chlortetracycline(228) *J. Am. Chem. Soc.* **79**, 4561, 4563, 4564 (1957)

1	2	3	4	5	6	7	8
226	*7-CHLORO-6-DEMETHYL-TETRACYCLINE *S. aureofaciens* (mutant)	Amphoteric. $C_{21}H_{21}N_2O_8Cl$. 1.5 H_2O (Sesquihydrate)	174-78 dec. (sesquih.)	230, 262, 267 (0.1*N* HCl)	-258 (0.1*N* H_2SO_4) (sesquih.)		Antibacterial activity similar to that of chlortetracycline (228) *J. Am. Chem. Soc.* **79**, 4561, 4563, 4564 (1957).
227	*BROMOTETRACYCLINE *Streptomyces* strains producing chlortetracycline	$C_{22}H_{22}N_2O_8$ Br	HCl : 235-40 dec. Free base: 170-72	227, 260, 370 (0.1*N* HCl) 225, 255, 285, 345 (0.25*N* NaOH)	-196 (0.1*N* HCl, 2°0); -205 (0.5% in 0.03*N* aq. HCl)		Antibacterial activity resembles that of chlortetracycline (228). *H. T.* **2.**
228	*CHLORTETRACYCLINE *S. aureofaciens*	Acicular to bladed, small. HCl : vitreous yellow, rhomboid Amphoteric. pka 3.30, 7.44, 9.27. $C_{22}H_{23}N_2O_8Cl$	168-69 uncor.	HCl : 230, 262, 367 (0.1*N* HCl)	-274.9 (MeOH, 23°)	In alc. $FeCl_3$ greenish brown by reflected light, reddish by transmitted light. Ppt. with picric acid, Reinecke's acid, ammonium molybdate. UV shifts in acids, alkali ; fluoresces in basic soln.	Inhibits gram +, gram— bacteria, rickettsiae, large viruses, some protozoa. Viruses include mouse pneumonitis, SF strain of human pneumonitis, feline pneumonitis, meningopneumonitis in ovo. *H. T.* **2.**
229	*TETRACYCLINE *Streptomyces* spp.	Orthorhombic, yellow (HCl) Amphoteric. pka 3.30, 7.68, 9.69 $C_{22}H_{24}N_2O_8$	170-73 dec. (anhydrous) HCl : 214	220, 268, 355 (0.1*N* HCl) 268, 363 (0.01M methanolic HCl) 246,372, (0.01M methanolic NaOH)	-239 (c, 1, MeOH, 25°)	Orange yellow soln. with Ehrlich in dil. aq. HCl, stable violet colour with H_2SO_4	Antibacterial spectrum similar to that of chlortetracycline (228). *H. T.* **2.**

1	2	3	4	5	6	7	8
230	***OXYTETRACYCLINE** *S. rimosus*	Pale yellow needles ; thick, hexagonal plates. Amphoteric; pka 3.27, 7.32, 9.11 $C_{22}H_{24}N_2O_9$	181-82 dec. (dihydrate) 190-94 dec. (HCl)	270, 370 (MeOH) 270, 359 (0.01*N* methanolic HCl) ; 245, 264, 375 (0.01 *N* methanolic NaOH)	-196.6 (dihydrate, c, 1 in 0.1*N* HCl, +26.5 (dihydrate, c, 1 MeOH)	Pos. $FeCl_3$, Pauly, Fehling, Friedel-Crafts. Complexes with inorg. salts.	Activity similar to that of chlortetracycline (228) *H. T.* **2.**
231	***2-ACETYL-2-DECARBOXAMIDO-OXYTETRACYCLINE** *S. rimosus* (mutant)	HCl : yellow cryst. pka 3.3, 7.1, 9.2 (aq. soln.) $C_{23}H_{25}NO_9$	HCl : 200-03 dec.	240sh, 277, 316sh, 357 (MeOH-0.01 *N* HCl) 237, 269, 376 (MeOH-0.1*N* NaOH)	HCl : —47 (c, 0.9 in 0.1*N* HCl)	Similar to those of oxytetracycline. I. R. peaks at 5.92, 6.1-6.3 μ	Antibacterial activity similar to that of oxytetracycline. *J. Am. Chem. Soc.* **82**, 5934 (1960)
		OTHER COMPOUNDS					
	ABIKOVIROMYCIN (LATUMCIDIN)	(*See* 75)					Inhibits western and eastern encephalitis virus. Poliomyelitis virus rendered non-infective when mixed with crude filtrates.
232	ACHROMOVIROMYCIN *S. achromogenes*					Related to abikoviromycin (75) and sarcidin. Extractable with ethyl acetate at pH 2.	Crude ppn. inhibits Japanese B encephalitis virus in mice. *Sarcina lutea* also inhibited. *H. T.* **2.**
	HYGROSCOPIN A	(*See* 79)					
	HYGROSCOPIN B	(*See* 89)					

1	2	3	4	5	6	7	8
233	NYBOMYCIN *Streptomyces* sp.	Light yellow small thin needles, rods depending on solv.	Darkens 330 without melting	266, 286 (conc. H_2SO_4) 222, 266, 286, 369	Inactive	Neg. Tollens, Benedict, neutral $KMnO_4$, *p*-dimethylaminobenzaldehyde, Ehrlich-Pauly; no reaction with Br in CCl_4	Inhibits bacteriophages, gram+ and gram—organisms. *H. T.* 2. *Antibiot. and Chemother.* **8**, 627, 631 (1958).
	CHRYSOMYCIN	(*See* 62)					
234	AKLAVIN *Streptomyces* sp.	Mixt. of closely related compds. Consists of non-basic water insol. moiety combined with some colourless water sol. basic structure	Picrate 168. Helianthate 197	228, 258, 288, 427			Active against gram+, acid fast bacteria, phage. Moderately fungicidal, and virucidal to eastern equine encephalitis, slightly to PR-8 influenza, and inhibits Y-SK polio virus in tissue culture. *J. Bact.* 72, 90, (1956).
235	ANTIBIOTIC X-465 *Streptomyces* sp.	$C_{32}H_{34\text{-}36}O_{14}$					Inhibits bacteriophage, actinomycetes, some bacteria. Intern. Congr. Pure and Appl. Chem. 14th, Zurich (1955) p. 233.
236	FLAVUCIDIN *Streptomyces* sp.	Colourless needles. $C_{34}H_{55}NO_9$	144-45	275 (MeOH)	+94 (c, 1 MeOH, 20°)	Neg. nibhydrin. Pos. nitroprusside, Molisch, diazo, *m*-dinitrobenzene reagents. Contains phenolic hydroxyl group.	Inhibits *M. flavus*, *Sarcina lutea*, influenza virus. *Ann. Rept. Takeda Res. Lab.* **17**, 19 (1958).
	ANTIBIOTIC 1-81d-1s	(*See* 110)					
237	VIROCIDIN *S. flavoreticuli*	Picrate : yellow needles. C 49.61, H 3.55, N 10.95	Browns above 120, gray at 200 then blackens. Explodes on rapid heating	240, 345		Picrate gives neg. $FeCl_3$, ninhydrin biuret, Sakaguchi, CS_2, Na-nitroprusside, Fehling, Molisch, Tollens, Nessler, Br_2, $KMnO_4$. Pure base unstable, neg. reactions as with picrate	Inhibits gram+, gram —bacteria and rabies virus. *J. Antibiot.* (*Jap.*) **11A** 128 (1958)

1	2	3	4	5	6	7	8
238	PHAGOCIDIN *Streptomyces* sp. resembling *S. antibioticus*	Light yellow cryst. Acidic.		235, 300 (1% MeOH)		Pos. Molisch (purple) Neg. Fehling, $FeCl_3$, Millon, biuret, Hopkins-Cole.	Inhibits phages. *J. Antibiot.* (*Jap.*) **9A**, 152, 157 (1956).
239	PHAGOSTATIN *Streptomyces* sp. resembling *S. bikiniensis*	Long needles. Neutral or weakly acidic.	Colour changes to yellowish at 125-30, dec. 184-90	No characteristic $U_{\lambda}V$. max.		Neg. ninhydrin, Fehling, biuret, Molisch, Hopkins-Cole, Millon, Ehrlich, $FeCl_3$.	Gram+ bacteria, *E. coli*, phage. *J. Antibiot.* (*Jap.*) **10A**, 74 (1957)
240	PHAGOLESSIN A-58 *S. griseus*	Light yellow amorphous, hygroscopic powder.				Neg. $FeCl_3$, biuret, Millon, ninhydrin, Lassaigne's nitrogen; reacts with picric acid, Reinecke's salt, methyl orange.	Inhibts gram+ bacteria, bacteriophage, some spirochaetes, protozoa. *In vitro* activity against viruses of vaccinia, herpes simplex, rabies; slight activity against Theiler and Lansing strains of poliomyelitis. Y-SK polio inhibited in tissue culture. *H. T.* **2.**
241	EHRLICHIN *Streptomyces* sp. resembling *S. lavendulae*					Dialyzes without loss of activity, resists tryptic digestion. Thermostable, acid labile.	Inhibits influenza B virus *in vivo*. *H. T.* **2**
242	CARDICIN *Nocardia* sp.	White cryst. Acidic					Inhibits Mycobacterium sp., some fungi. Active against bacteriophage, and strains PR-8 and FM-1 influenza virus in chick embryo. *H. T.* **2**

1	2	3	4	5	6	7	8
243	CERULOMYCIN *S. coerulescens*	Colourless plates. Contains no N, S.	181-83	240, 260, 380, 400, 420 (approx.)			Chemotherapeutic action in mice infected with influenza virus. Suppresses actinophage. *Antibiotiki* 2, No. 6, 16 (1957) ; *Antibiotics* (*Trans.*) **4**, 715 (1959)
244	HELIOMYCIN *S. flavochromogenes* var. *heliomycini* (mycelium)	Cryst. product has 2 components, one of which is the product of inactivation. Contains no N, S, P.	Ac. deriv. 265-67	269, 290, 320, 340, 370, 460, 515 (alc. soln.)		U. V. and I. R. spectra indicate presence of carbonyl and HO groups, and similarities to tropolones. Enol acetate group indicated in the acetyl deriv. Colour reactions characteristic of phenol and quinone.	Effective, in large doses, against smallpox vaccine virus in chick embryo. Neutralizes influenza A *in vitro* and effective in mice. Also inhibits TMV *Antibiotiki* **3**, No. 2, 29 (1958) ; *Zhur. Obshchei Khim* **29**, 3504 (1959) ; *Antibiotics* (*Trans.*) **4**, 75 (1959)
245	CYTOVIRIN *Streptomyces* sp.						Effective against southern bean mosaic virus, and TMV *Plant Dis. Rept.* **41**, 576 (1957) ; *Phytopath.* **48**, 344 (1958) ; *ibid.* 49, 502 (1959)

INDEX TO MELTING/DECOMPOSITION POINTS (RANGE IN °C)

M. P.	50—55	55—60	60—65	65—70	70—75	75—80	80—85	85—90	90—95	95—100
S. No.		217*								
M. P.	100—05	105—10	110—15	115—20	120—25	125—30	130—35	135—40	140—45	145—50
S. No.	216	219	86		75				106 236	106
M. P.	150—55	155—60	160—65	165—70	170—75	175—80	180—85	185—90	190—95	195—200
S. No.	209	113		224* 228 234*	114 227 229	181 226*	230 243	220* 239	230*	234*
M. P.	200—05	205—10	210—15	215—20	220—25	225—30	230—35	235—40	240—45	245—50
S. No.	231	225*	229*		220*			227*		
M. P.	250—55	255—60	260—65	265—70	270—75	275—80	280—85	285—90	290—95	295—300
S. No.		62	211*	244*				218*		

Remarks on asterisk-marked numbers : (211) HCl, sulphate. (217) m. p. 47-67. (218) Sulphate. (220) Helianthate 215-25 dec. ; reineckate 187-90 dec. (224) Reineckate. (225) HCl hemihydrate. (226) Sesquihydrate. (227) HCl. (229) HCl. (230) HCl. (231) HCl. (234) Picrate 168 ; helianthate 197. (244) Ac. deriv.

INDEX TO U. V. ABSORPTION MAXIMA

λmax (mμ)	0	1	2	3	4	5	6	7	8	9
210	218*	218			219					218
220	229	233				227		227	234	
230	106 226 228			75 89 233		75 238 79 113 114		231		
240	237 243					75 106 230	229	62		
250					219	227			114 234	113
260	227 243		226 228		230		233	226	181 229	231 244
270	230		219			181 236		231	209	
280		222				227	219 233	62	234	
290	75 83 244				113 114					
300	238									
310										
320	106 244			219						
330							75		75	
340	244					227 237		106		
350						229		231		230
360				229				228		
370	227 230 244		229			230	231			
380	243									
390						62				
400	243									
410										

*205 ; 207

λmax (mμ)	0	1	2	3	4	5	6	7	8	9
420	243							234		
430										
440										
450										
460	244									
470				113 114						
480								113	114	
490								113 114	213	213
500	213									
510						244			113	114
520										
530	213		114	113						

FORMULA INDEX

Formula	Melting point (°C)	S. No. of Antibiotic
$C_9H_{18}N_4O$	Sulphate : 284-90 (sealed tube)	218
$C_{11}H_{12}N_2O_5Cl_2$	149.7-150.7 corr.	209
$C_{11}H_{13}NO_2$	120-25 dec.	75
$C_{14}H_{21}NO_4$	93-94, 96-98	83
$C_{14}H_{21}NO_4$	98-105	216
$C_{15}H_{20}N_6O_3$	HCl : 167-72 dec.	179
$C_{15}H_{23}NO_4$	109 - 116, 120	86
$C_{15}H_{28}N_2O_3$	B. P0.008 70	89
$C_{17}H_{34}N_{10}O_5$	HCl : 265 dec. Sulphate : 263 dec.	211
$C_{21}H_{21}N_2O_8Cl$	174-78 dec. for sesquihydrate	226
$C_{21}H_{22}N_2O_8$	203-09 for HCl hemihydrate	225
$C_{22}H_{20}O_7$	255-60 (microblock)	62
$C_{22}H_{23}N_2O_8Br$	170-72	227
$C_{22}H_{23}N_2O_8Cl$	168-69 uncor.	228
$C_{22}H_{24}N_2O_8$	170-73 (anhydrous)	229
$C_{22}H_{24}N_2O_8$	181-82 dec. (dihydrate)	230
$C_{22}H_{29}N_7O_5$	175.5-177 uncor.	181
$C_{22\text{-}24}H_{32\text{-}34}O_{8\text{-}9}$	..	213
$C_{23}H_{25}NO_9$	HCl : 200-03 dec.	231
$C_{23}H_{29}NO_{12}$	105-109; dec. gradually above 160	219
$C_{28}H_{40}N_2O_9$	139-140, 149.8-150.2	106
$C_{31}H_{34\text{-}36}O_{14}$	..	235
$C_{34}H_{55}NO_9$	144-45	236
$C_{38}H_{63\text{-}65}NO_{12}$	140-41	110
$C_{43\text{-}45}H_{57\text{-}61}NO_8$	155-58, solidifying at 160-88 in rosette needles which disappear above 249	113
$C_{43\text{-}45}H_{57\text{-}61}NO_{18}$	168-78, solidifying on further heating, disappears at 240-43. 180 (capillary)	114

HINDUSTAN ANTIBIOTICS

Bulletin

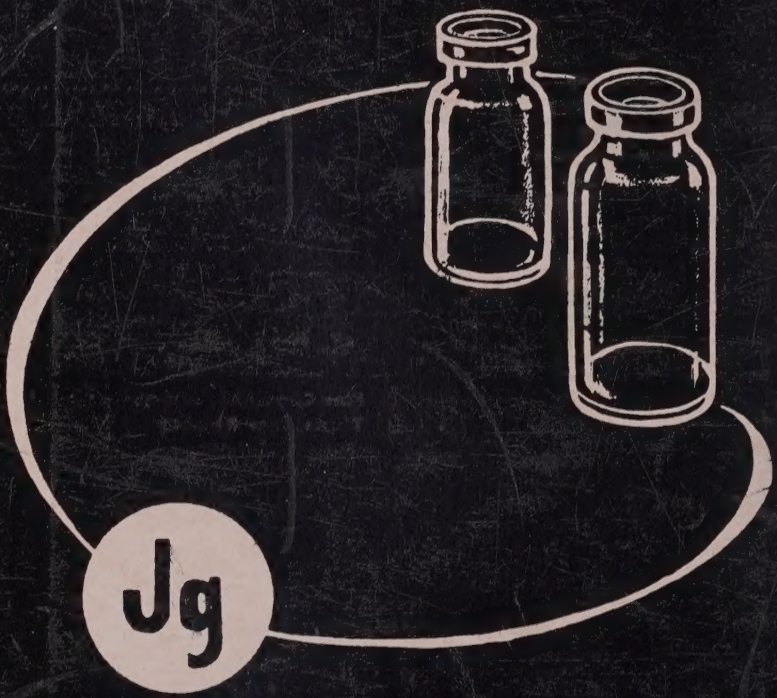
VOLUME 3

1960-61

HINDUSTAN ANTIBIOTICS LTD.
PIMPRI.

GENERAL INDEX

Edited by Dr. M. J. Thirumalachar and Published by Shantikumar T. Raja, B.A., LL.B. Bar-at-Law, Hindustan Antibiotics Ltd., Pimpri (near Poona). Printed by Rev. Theodore A. Pereira at the Examiner Press, Bombay—1.



JG GLASS INDUSTRIES,

PIMPRI (NEAR POONA)